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International News on Fats, Oils, and Related Materials

Changing traits without changing DNA

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A new kind of plant breeding

Rebecca Guenard

Armies of caterpillars and worms will annihilate unprotected corn crops, but proteins from a naturally occurring bacterium kill the pests. For decades, the bacterium, known as *Bacillus thuringiensis*, or Bt for short, has been a reliable organic pesticide spray. However, as a spray application, Bt faces challenges. Its proteins decompose in the sun and wash away in the rain, which led researchers to invent a more reliable way to use the bacterium to protect corn.

- **Researchers can breed traits into plants without changing their DNA sequence.**
- **The epigenome is a set of molecules that swarm the genome and determine the activity of genes based on environmental influences.**
- **If researchers can identify and select for epigenetic traits with long-term heritability, they could endow agricultural crops with the resilience to withstand an unpredictable environment caused by climate change.**
- **Two start-ups have already acquired investments and hope to capitalize on this technology.**

In the mid-1990s, scientists genetically engineered the DNA of corn plants to include genes from Bt. The genetically modified plant had built-in protection from caterpillars and other insect larva. The crop was so economically and environmentally successful that most of corn grown in the United States is Bt-corn. According to the US Food and Drug Administration, in 2018, 92% of corn planted in the United States was genetically modified.

In the decades since the development of genetically modified crops, consumer and environmental groups have voiced concerns about their use. Editing techniques have evolved from inserting foreign DNA with large altered sequences into a plant's genome to much more precise modifications that alter just a few DNA letters without the need to transplant genes from another species. Now researchers are considering whether they can develop desirable traits in crops without changing even one letter in the code.

"Classical genetics tools alone cannot help us solve complex problems, like climate change," says Dragana Miladinović, a plant biologist at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. "Researchers observed that a plant's response to environmental stresses could be controlled using more modern tools."

A DNA sequence functions beyond its collection of genes. Whether genes operate or remain dormant depends on the molecules surrounding them. Researchers see potential in breeding desired traits into plants by manipulating these molecular switches that turn genes off or on while leaving a DNA sequence unaltered (Fig. 1).

EPIGENETICS

Heredity is not determined solely by the genes coded in DNA. The molecular environment around a gene can also be passed down to offspring as cells multiply through mitosis or meiosis. Researchers currently label this type of heredity, epigenetics.

Pull DNA from any cell within an organism, and they will all contain the exact same genetic code. The instructions to make a complete organism is contained in every cell, but each cell has a unique job within an organism. Epigenetics gives cells their individuality. When a group of cells form the stalk of a plant, for example, the genes that control flowering are silenced within the DNA of those stalk cells, since flowering is meant to occur elsewhere.

A gene is silenced or activated depending on whether transcription molecules can access that stretch of DNA to read

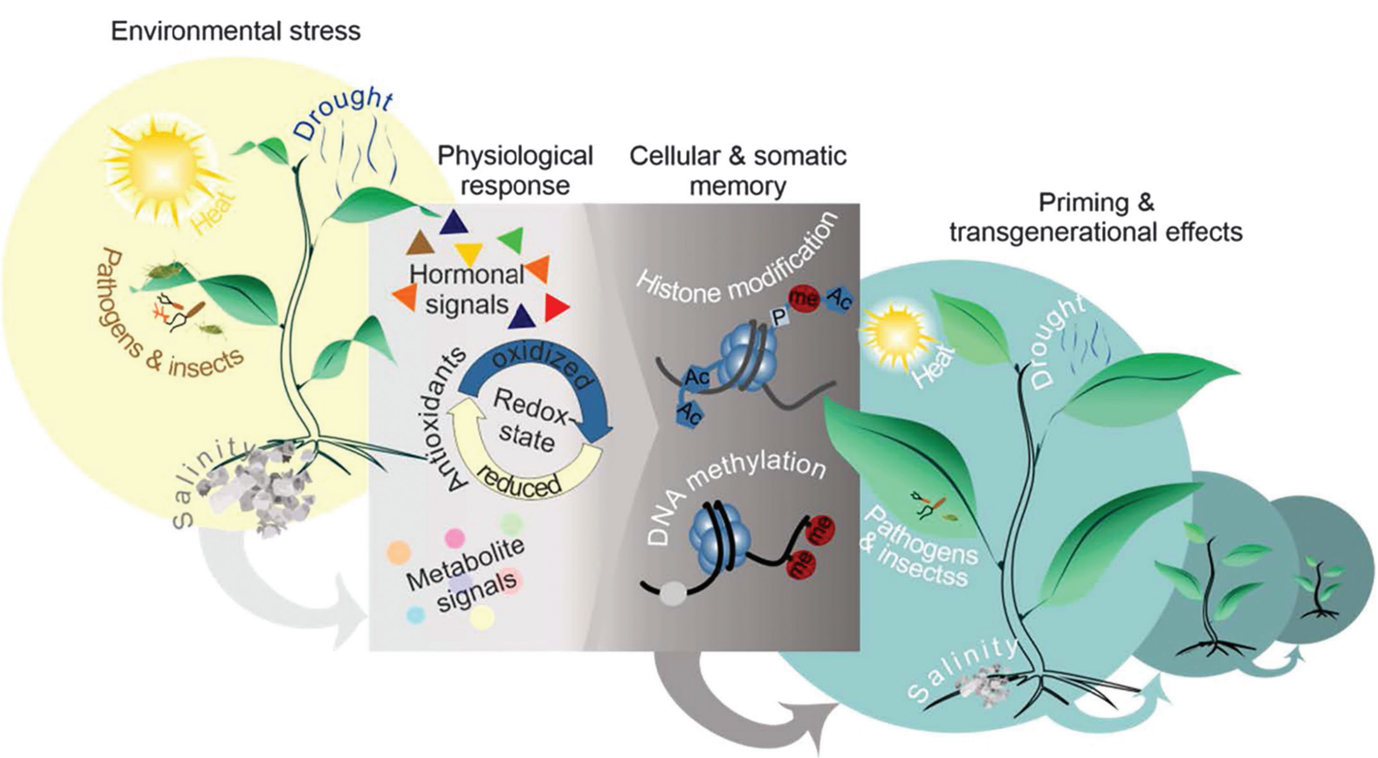

FIG. 1. Physical and environmental threats to crops leave a memory in the plant through changes in the epigenome. Researchers are taking control of these changes to breed more climate-tolerant crops. Source: Mladenov, V., *et al.*, *Int. J. Mol. Sci.*, *22*, 13, 7118, 2021.

it. The ways parts of the code are blocked or exposed varies. A gene may be silenced when a series of enzymes create opposing charges that lead stretches of DNA to coil tightly around protein spools, called histones. By contrast, loosely wound DNA means an active gene. Acetyl groups astride a gene cause the DNA to be repelled from the histone because of similar charges. Whereas proteins or methyl groups restrict access to genes by physically surrounding them so they cannot be read.

The variety of modifications available to tweak the genetic code gives an organism the versatility to adjust to inputs from the environment. A plant that survives drought could potentially pass along methylation patterns that increase drought tolerance in its offspring. Unlike typical patterns of inheritance, such epigenetic states are fluid. The activity of a gene can be influenced by the experience of a parent and passed on to later generations.

Researchers are in the process of categorizing the effects of specific instances of altered epigenetic states in plants and how they contribute to cellular function. Once they understand a plant's epigenetics they can use it to breed in traits that have commercial benefits, like increased protein or fatty acid content. Scientists are also hoping this type of breeding will contribute to a sustainable food supply by making plants more robust to changes in climate.

"Agriculture is facing more and more problems. Crops need to produce more with less input from humans, due to regulatory and consumer constraints," says Miladinović. She is part of a collaborative, multidisciplinary group of scientists from 22 countries gathering and sharing findings on plant gene expression to solve the problem of making future food production adaptable to climate change.

"The application of epigenetics is no different than applying genetics in breeding, you are just using a different mechanism," she says.

ARTIFICIAL EPIGENETICS

After observing the methylation changes that occur naturally in plants, researchers were interested in the effects of inducing epigenetic changes in the laboratory. They have since determined that epigenetic alterations can be performed in a number of ways, including abiotic stress, tissue culture, RNA-based methods, and grafting. The following paragraphs describe some of their preliminary research efforts.

Abiotic stress in plants generally includes drought, heat, cold, and salinity. The exact mechanism for how abiotic stress affects the epigenome is not yet completely clear. Experiments show that even the application of phytohormones associated with these kinds of stresses modify the plant epigenome and lead to lasting variation. Collectively, studies show that plants do not exhibit the same response to a particular stress. Each plant responds with different methylation effects. More research is needed to identify the genome-wide epigenetic changes that result from exposure to abiotic stress or the phytohormones it causes in order to determine its most beneficial manipulation (Table 1).

Plant regeneration and propagation were once believed to produce a plant identical to the donor; however, researchers discovered that during tissue culture cells could erase some of their epigenetic markers. They observed that the hormones used in the growing medium can lead to a regenerated plant with unique epigenetic profiles. A complete assessment of these changes still needs to be explored.

TABLE. 1. A few examples of epigenetic mechanisms involved in crop response to different abiotic stress.
Source: Varotto, S., *et al.*, *J Exp Bot, 71*, 17, 5223–5236, 2020.

Crop	Abiotic stress	Epigenetic mechanism(s)	Reference
Soybean	Drought	miR1514a modulation on a NAC transcription factor transcript	Sosa-Valencia, *et al.* (2017a)
		Up-regulation of isomiRNAs	Kulcheski, *et al.* (2011)
	Heat	Hypomethylation of cytosine	Hossain, *et al.* (2017)
Pea	Drought	Hypermethylation of cytosine residues	
Chickpea	Drought	Accumulation of miR408 transcripts	Hajyzadeh, *et al.* (2015)
	Drought + Salinity	Accumulation of miRNAs at root apex	Khandal, *et al.* (2017)
Cowpea	Drought	Increase of P5CS transcripts and very low expression of vun-miR5021 and vun-miR156b-3p	Shui, *et al.* (2013)
Bean	Drought	Dicistronic arrangement of miR398a and miR2119	De la Rosa, *et al.* (2019)
Faba bean	Drought	Increased DNA demethylation	Abid, *et al.* (2017)
Alfalfa	Drought	Overexpression of miR156	Arshad, *et al.* (2018)
Rapeseed	Heat	Increased DNA demethylation	Gao, *et al.* (2014)
	Salinity	Increased DNA demethylation	Marconi, *et al.* (2013)

Bacterial and fungal pathogens may also affect a plant's epigenome. Pathogens transfer RNA to their host to silence the host's defense genes. It is possible that pathogen RNA could also disrupt the host's DNA methylation, but so far no one has reported this type of experimentally induced epigenetic alteration in plants.

Grafting the shoot of one plant onto another rooted plant originated as a means of harnessing the robustness of that plant and passing it on to the shoot. In grafting studies on a variety of plant species, researchers have determined that the technique also alters DNA methylation of the shoot. The graft-induced epigenetic changes remain intact even after pollination and the formation of progeny. Some researchers see grafting as the optimal way to manipulate the epigenome of a crop.

Of course, scientists have methods to directly alter the places within the genome where the epigenetic instructions are written. In contrast to the methods mentioned above, which affect the global genome, gene-specific insertions and deletions that control DNA methylation or histone interaction would be a more precise way to conduct epigenetic breeding. Although potentially effective, such human-guided gene-editing methods are not accepted in many countries.

Finally, researchers have experimented with externally applying RNA molecules to instruct specific genes to make epigenetic changes. They found that spraying foliage or soaking roots with RNA that promotes methylation triggered an increase in DNA methylation. More research is needed to improve RNA delivery, but the researchers are hopeful this type of fine-tuned epigenetic breeding would fall outside of current gene-editing regulations.

LASTING CHANGE

The range of examples given above show the complexity of options available when considering how best to use epigenetics in agriculture. For instance, some chemicals applied to seedlings are taken up by cells and incorporated into their DNA as they replicate. However, the epigenome is typically restored to its original state when chemical treatment is removed, rendering this method impractical for crop applications. To harness the epigenome, researchers must identify changes that transmit across generations.

In experiments performed on the model plant species *Arabidopsis*, they found that the plant carried an epigenetic memory from its ancestors. Over three generations, in accordance with a moderate ambient temperature increase, the plant produced less of an RNA involved in silencing a gene. After three generations, the effect's strength declined. The researchers observed more enduring epigenetic memory associated with pathogen or UV light-induced stress. Such abiotic stressors may even induce heritable changes leading to more environmentally robust phenotypes.

Taking epigenetic knowledge from the laboratory to the field is the next step in realizing the potential of this breeding strategy. The epigenomes of critical staple crops like rice, corn, wheat, and barely have been picked over in recent years to identify epigenetic memory markers on stress-responsive genes that could be useful. AOCS-relevant crops that have been investigated include rapeseed and, to a lesser extent, soybeans.

Since rapeseed is a recently domesticated hybrid crop, it has yet to develop extensive genetic diversity. Less diversity in rapeseed DNA provides an opportunity for greater influence from epigenetic changes. Studies on DNA methylation of rapeseed genes indicate a sensitivity to heat and salinity. Overall, it seems that rapeseed genotypes with less methylation also have higher stress tolerance. Hence, crop varieties with this epigenetic profile could be agriculturally more resilient.

Researchers identified the epigenetic component that determines how efficiently a rapeseed plant uses energy. The way the plant uses energy predicts its vigor and yield. Artificial selection of the plants with this desired epigenetic trait became heritable upon self-fertilization. Furthermore, hybrids from parental lines selected for high energy use efficiencies had a 5% yield increase.

"According to data available so far, recurrent selection seems to be the best tool for introducing epigenetic traits in crops," says Miladinović. She cautions that, aside from rapeseed, most of this research has been carried out on model plants and not yet applied to crops. "As the genome becomes more complex, it is more difficult to control some things," she says. This challenge has not deterred a few entrepreneurs.

COMMERCIALIZING EPIGENETIC BREEDING

According to *Forbes*, the overall epigenetics market will grow to $35 billion by 2028 (https://tinyurl.com/44d32hfr). Most of that value will likely come from diagnostic and treatment products for human health, but crop enhancement companies are also gaining traction.

This year, a start-up company called Sound Agriculture, established in Emeryville, California in 2013, announced it had secured $45 million in funding that it will use to support two new platforms for creating climate-resilient crops (https://tinyurl.com/8z5abt8j). The first focuses on adjusting a soil's microbiome to increase the nutrient uptake efficiency of a crop. The second is aimed at developing specific heritable traits through altered plant epigenetics.

On-demand breeding, as it is called, accelerates plant trait development 10 times faster than gene editing, according to the company's website (https://www.sound.ag/). The company hopes to identify and optimize traits that make plants more resilient with respect to climate change, diseases, and reduced chemical use. They are also considering traits that optimize nutrition, appearance, and flavor. Sound claims that breeders can progress from concept to a plantlet with a new trait in 15 days, compared to the 150 days needed for gene editing.

A similar company was founded by plant biologist Sally Mackenzie, currently at Pennsylvania State University in University Park, Pennsylvania, USA. Mackenzie discovered that a plant protein, known as MSH1, encodes for processes that determine DNA binding and recombination suppression (Fig.2, page 10). She argues that evidence indicates MSH1

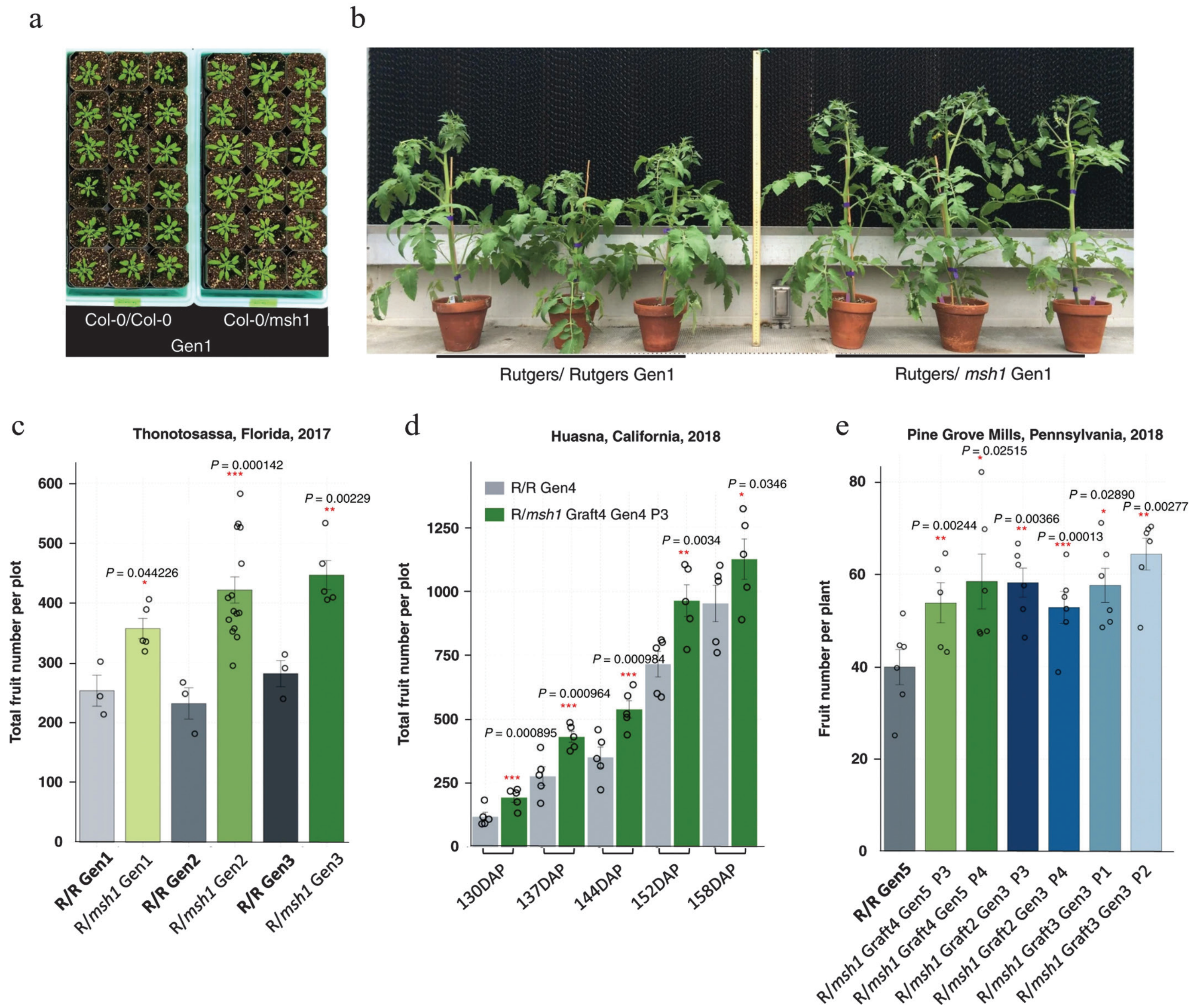

FIG. 2. a. Seedlings from grafted Arabidopsis plants comparing a control graft (left) with a graft from a plant containing a mutation on the msh1 gene. Research shows mutations on this gene trigger epigenetic reprogramming in the plant. b-e. Examples of greater vigor and seed yield for msh1 mutated tomato plants over multiple generations in different field locations. Source: Kundariya, H., *et al.*, *Nat. Comm.*, *11*, 5343, 2020.

is environmentally adaptive, so breeders can direct how a plant adjusts to its environment by targeting methylation on the gene that encodes MSH1 (http://dx.doi.org/10.1098/rstb.2019.0182).

Epicrop Technologies was established in 2013 in Lincoln, Nebraska, where Mackenzie was previously a professor at the University of Nebraska-Lincoln. After receiving funding from TechAccel, the company launched platforms to use epigenetics to improve two crops: strawberries and canola. They hope to improve the strawberry's disease resistance and environmental range, while their research efforts for canola focus on yield.

The future of epigenomic breeding has many exciting prospects, according to Miladinović. The involvement of a variety of scientists using a range of tools means a quicker pace of discovery. A combination of epigenetics, genomics, and other omics tools with high-throughput analytics tools and artificial intelligence allows more data to be gathered and evaluated faster.

Miladinović says that since the concept for epigenetic breeding is still unproven in some crops, there is no way to know if desired traits will be heritable over many generations of a plant. "We still need to go case by case, crop by crop, and trait by trait," she says. Even with classical breeding there are some traits that are easier to maintain than others. She says researchers will have to be careful to first identify traits with potential for heritable change.

One limitation of epigenetics is made obvious by our earlier example of Bt-corn. Scientists cannot add or remove any information from a plant's genetic code. This means that incorporating the gene from a natural pesticide into a crop plant is off-limits in the epigenetic breeding playbook. If a gene responsible for some self-protective measure to ward off pests is not already present in the genome, there is no epigenetic course of action to address such a threat.

Another issue is that a plant epigenetically bred to resist heat may be left vulnerable if exposed to flooding. Miladinović says epigenetics is not going to produce crop varieties that can be planted anywhere. More likely, the discipline will assist with the new trend of agroecology, where crops are specialized for specific regions. "Farmers will not grow the same variety for different countries around the world, like they do now with seeds from a big corporation," she says. Instead, farmers will select crops with one or two traits best suited for their agro-ecological area.

"We have discovered something beyond the classical genetics alphabet," says Miladinović. "We have the potential to create a plant genotype that is really adaptive to a certain climate or a certain soil. This new breeding tool opens new ways to explore genetic diversity so we can assure stability of crops production as it faces environmental change."

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Giants of the past: Frank Denby Gunstone— Father of Lipid Chemistry (1923–2021)

Marcel S.F. Lie Ken Jie

It was a beautifully calm and sunny day in St. Andrews, when my wife (Rosita) and I visited Frank for the last time on April 30, 2019. We had brought along a parcel containing a selection of fine chocolates, bonbons, cookies, and potato crisps, but I wondered whether Frank was able or even allowed to enjoy these little gifts. The thought of my 95-year-old mentor "sequestered" at a care home in St. Andrews, Fife, Scotland, made me envisage him lying in bed with a caregiver or nurse hovering over him. How wrong I was.

When we entered his ensuite room, he was seated in a comfortable armchair near the window, an iPad in his hands. He was so pleased to see us, and we to see him. Hearing him speak in his usual soft tone with a touch of a Midland or Liverpool lilt reminded me of how pleasant it was to hear his voice once more. It was 54 years ago (1965) when Frank took me under his wings. At 95, his mind was clear and lucid, and his thinking was sharp as ever. His hearing was hardly affected. His "trademark" patience in letting us tell him about our journey to Scotland and share news from our families without an interruption was "classic". He inquired about my teaching involvement with the universities in Hong Kong, only to be disappointed that I too had retired from academic life two years ago. We were both retirees. He smiled and shrugged, as if to mean that we were both still capable and able to serve. I could sense that the "fire in his belly" as a teacher, researcher, and writer had remained unextinguished since his retirement. He confessed that he missed his literature searches and writings very much and often felt "bored".

He then told us about his life at the care home, where he had moved after his wife, Eleanor, passed away the year before. His only "complaint" was that he could not find a resident in the place with whom he could have a meaningful discussion. We shared a laugh, but he admitted that he was very comfortable and well cared for. When the weather was uninviting, he kept in shape by making a daily round of the corridors; on sunny days, he spent an hour or two on the porch soaking up the sunshine. Members of his large four-generation family visited him frequently, and he was in constant touch with them via his iPad, email, and FaceTime.

Our visit soon came to an end, and I promised my "shi fu" (Master) that I would be back next year to visit him again. Sadly, the pandemic did not allow me to do so. Frank passed away on October 30, 2021, at the age of 98.

In the spring of 2014, the organizers of the 7th Workshop on Fats and Oils as Renewable Feedstock for the Chemical Industry held in Karlsruhe, Germany—Professor J. Metzger, Professor M.A.R. Meier, and Dr. U. Biermann—invited me to deliver a special lecture to celebrate Frank's 90th birthday and decision to finally "hang up his spurs". My presentation, *Frank Gunstone—Teacher, Researcher and Writer*, was subsequently redacted and published in the *European Journal of Lipid Science and Technology* (*Eur. J. Lipid Sci. Technol. 117*: 135–140, 2015). It is now my bittersweet privilege to share some details of Frank's life and legacy in this tribute.

FORMATIVE YEARS

Frank was born on October 27, 1923, in Chadderton (a small town eight miles north of Manchester, England, UK), which had one of the largest cotton mills in the United Kingdom at that time. The mill was driven by a Parson steam turbine, which drove the wheels by ropes. In 1936, the Gunstone family moved to Liverpool. Frank completed his secondary schooling in 1940 and was admitted to the University of Liverpool to read chemistry. He graduated three years later with a First Class honors degree in chemistry.

With the Second World War still raging, he was "invited" (drafted) to work for a PhD degree under the supervision of Professor T.P. Hilditch, the Head of the Department of Chemistry of the University of Liverpool. Hilditch was at the time a world authority in fats and oils and was regarded by chemists as the "Father of Fats and Oil Chemistry". To contribute to the war effort, Frank's research was to focus on the preservation of edible oils and to search for potential new sources of fats and oils other than those available at the time. His first challenge was to investigate the chemistry of the autoxidation of methyl oleate (18:1), linoleate (18:2), and linolenate (18:3). The aim was to provide a basic chemical view to the question as to why some edible oils were more prone to spoilage (becoming rancid) than others, when such oils were left exposed to air. His second task was to study the chemical composition of the Australian lumbang oil (*Aleurites moluccanus*) and on an African drying oil from the seeds of *Tetracarpidium conophorum*.

Frank relied on well-established chemical techniques of the time, when chromatography, spectroscopy, and spectrometry were terms yet unheard. He relied on fractional crystallization of salts of fatty acids (using cooling baths as low as –50°C) to separate and isolate the different fatty acids of the oil. Reactions (such as the hydrolysis of seed oils into fatty acids) were often conducted with the starting material (seed oils) on a scale exceeding 100 g. It was what was often referred to as "bucket chemistry". Each isolated fraction underwent further separation by repeated fractional crystallization. When an isolated fraction was deemed as being pure (a single fatty acid), the structure of the fatty acid (in the pure form or as a chemical derivative) was identified by its melting point and confirmed by taking a "mixed melting point" with an authentic sample. How else? Readers, there is no need to roll your eyeballs in disbelief. If you have never done a melting point determination, please give it a try. It is an art!

Another technique was fractional distillation of the methyl esters of fatty acids using a Perkin triangle under reduced pressure. Each fraction, whether obtained by fractional crystallization or by reduced pressure distillation, had its iodine and saponification values determined to gain a quick insight of the fatty acid components present in the isolated fraction. To unravel the fatty acid composition of a seed oil (assuming the normal mix of saturated and unsaturated fatty acid), it took at least three weeks to complete—even with careful planning to make the best use of time. (Laboratory glass apparatus used in the 1950s were "cork fit" (not of the Quickfit type). He was awarded his PhD degree in 1946.

> "*In the '80s, Frank had the idea of putting together* The Lipid Handbook. *(I always thought the name ironic, since the editions ran to between 850 and 1,447 pages!). He asked me to join him for the biochemical part of all three editions. I must say that he was a fantastic person to collaborate with—always well-informed, full of ideas, and a master of organization. But, despite his Scottish home, I never persuaded him to join me with a malt whisky!*"
>
> **—JOHN HARWOOD, a professor in the School of Biosciences at Cardiff University, Wales, and co-author of *The Lipid Handbook***

THE TEACHER AND ADMINISTRATOR

Frank was offered a lectureship in chemistry at the University of Glasgow, Scotland, after obtaining his PhD degree from Liverpool. He proved himself to be a great teacher right from the start. It was during his tenure at the University of Glasgow, where he first included a session on the chemistry of fats and oils to his students. The recommended textbooks in Organic Chemistry had literally nothing on this subject. Fatty acid chemistry was a "forgotten field of Organic Chemistry". With his knowledge and practical experience on fats and oil, Frank made a start on his classic book: *An Introduction to the Chemistry of Fats and Fatty Acids,* which he published in 1958 with later editions refreshed in 1967, 1996, and 2004. Frank stayed in Glasgow for eight years (1946–1954) before moving to St. Andrews.

On his arrival in St. Andrews, Professor John Read (Head of the Department of Chemistry at the University of St. Andrews) invited him to help revise the 3rd edition of his book, *A Textbook of Organic Chemistry*, which was first published in 1926. Frank's contribution allowed the 4th edition of this textbook to appear in print in 1958, the same year Frank's first book on fatty acid chemistry was published. In the following decade, Frank wrote numerous textbooks for undergraduate teaching, which included a series on single topics in Organic Chemistry using the concept of "programmed learning", a textbook on stereochemistry, and on practical organic chemistry with colleagues in the Department. He was an inspiration to his colleagues and a most revered teacher in the Department.

Frank's ability to associate with and reach out to his colleagues made him a wanted leader of the Faculty of Science. He was elected Pro-Dean (1970–1973) and thereafter served as the Dean of Science for another three years (1973–1976). His administrative abilities and his dedication to the University caught the attention of the Principal (Vice Chancellor) of the University. Frank was appointed Vice-Principal of the University with the portfolio to develop research and teaching at the University in the next four years (1977–1981). The heavy administrative load was on top of his normal teaching duties and forefront research work. Where he got the energy to fulfill all these duties remains a mystery to me. Frank laid the foundation for quality teaching and research excellence in the University and lived to witness the University of St. Andrews become the top university in the United Kingdom this year (2021) for student satisfaction and teaching quality as per The Times and Sunday Times Good University Guide.

THE RESEARCHER

Glasgow (1946–1954)

The postwar years were lean ones for academic institutions, and chemical research was moving at snail-speed. Frank's first publication (1952) after arriving in Glasgow was on *Strophanthus sarmentosus* seed oil, where he described the discovery of the hitherto unknown *iso*-ricinoleic acid [9-OH,12c-18:1]. Applying the practical skill he had acquired in Liverpool under Hilditch (low-temperature crystallization and reduced pressure distillation), he obtained a pure sample of the acetylated methyl ester of a C18 hydroxy-monoenoic acid (6.6% of the total fatty acids of the seed oil). The structure of the novel fatty acid was confirmed by chemical elucidation as follows.

Hydrogenation of the novel C18 hydroxy-monoenoic acid fraction from *S. sarmentosus* seed oil furnished the corresponding saturated C18 hydroxy fatty acid, which to his surprise had a different melting property than that of 12-hydroxy-stearic acid (obtained from ricinoleic acid by hydrogenation). He was quick to realize that the difference in the melting point was possibly due to the hydroxy group being at another position of the fatty acid chain or due to a difference in the length of the alkyl chain. To determine the position of the hydroxyl group in the alkyl chain, the saturated C18 hydroxy fatty acid derived from *S. sarmentosus* was oxidized to the oxo derivative and converted to the corresponding oxime derivative. A Beckmann rearrangement reaction followed, which cleaved the alkyl chain to yield two amido containing intermediates. Hydrolysis of the amido intermediates furnished decanoic acid and azelaic acid (C9 diacid). Both decanoic acid and azelaic acid were confirmed by their melting points (and mixed melting points) with authentic samples and derivatives of these compounds. From these results, the position of the hydroxyl function was confirmed to be located at the C-9 position of an 18 carbon alkyl chain.

To locate the position of the unsaturated center in the C18 hydroxy-monoenoic acid, the unsaturated center was subjected to an oxidation cleavage reaction, which yielded a mixture of hexanoic acid and a C-12 γ-lactone fatty acid. These products were successfully identified and confirmed by comparing their melting points with authentic samples and with derivatives obtained from hexanoic acid and that of the C-12 γ-lactone. From these chemical deductions, the double bond position was therefore located between the C-12/C-13 positions of the alkyl chain. The structure of the novel C18 hydroxy-monenoic acid was therefore the 9-hydroxy-12-*cis*-octadecenoic acid (*iso*-ricinoleic acid).

Two years later (1954), Frank made another major discovery. He came across another novel and unique epoxy C18 monoenoic acid in the seed oil of *Vernonia anthelmintica*. As no modern state-of-the-art analytical technique was available, the discovery was not only a showpiece of his vast knowledge of organic and lipid chemistry, but it also demonstrated his great practical skill and keen observation power he possessed to query and challenge "unexpected" experimental results. Quoting Frank from his correspondence to me, he wrote: "I discovered the novel acid by the cumbersome chemical procedures required at the time. Had measurements been taken by a less experienced person (research student) the observation and its significance might have been overlooked."

He first suspected something unusual while studying the saponification values (SE) of the isolated epoxy fatty acid fraction of the seed oil. The SE value of the free fatty acid (from alkaline hydrolysis) was found to be 314.1, while the SE values determined for the corresponding methyl esters depended very much on the catalysts used in the preparation of the methyl esters. The methyl ester that was obtained from methanol and anhydrous HCl (g) had an SE value of 255.6, while the methyl ester derived from methanol in concentrated H_2SO_4 gave an SE value of 350.7. The discrepancies in the SE values were unexpected for the methyl esters, which were anticipated to be similar but 14 units (COOMe vs COOH) higher than 314.1 for the free fatty acid. These differing SE values suggested that the methyl ester prepared by methanol and HCl (g) contained a reactive chlorine atom. Following this lead, Frank went on to describe in detail the cumbersome but elegant chemical elucidation which led to the identification of a novel and unique epoxy fatty acid [*viz.* 12,13-epoxy-9c-18:1 (vernolic acid)] in the seed oil of *Vernonia anthelmintica*. In an email to me in 2014, he noted: "Today it would take me less than a week to isolate and confirm the structure of vernolic acid using chromatographic methods, nuclear magnetic resonance spectroscopy, and mass spectrometry."

By the end of the 1950s some other novel hydroxy, acetylenic, and oxo fatty acids from seed oils were discovered, such

> *"I first encountered Frank in his lectures to undergraduates in 1957, when I was greatly impressed by the clarity and organization of his presentations and the personal interest he took in his tutorials. When it came time to consider a PhD degree, it was the person rather than the topic that was the main factor in my decision. Three successful years later, I was happy to take his advice and apply to Ralph Holman at the Hormel Institute for a post-doctoral fellowship, and when that ended Frank made a temporary place available in his lab for me while I looked for a permanent position. While I was subsequently employed at the Hannah Research Institute in Ayr, Frank commissioned me to write my first independent review articles and encouraged my further literary ambitions. He was always available to discuss lipid science and act as a mentor."*
>
> **—BILL CHRISTIE, former student, co-founder of Mylnefield Lipid Analysis, and originator of the AOCS Lipid Library®; excerpted from *Lipid Matters* (https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/info/blog.htm)**

as kamlolenic acid [18-OH 9,11,13-18:3], santalbic acid [9a,11t-18:2], and isanic acid [17e, 9a,11a-18:3].

St. Andrews (1954–2013)

Frank moved to St. Andrews in 1954, after a period of eight years in Glasgow. At the turn of the 1950s, the world was introduced to a novel analytical technique: Martin and Synge were awarded the 1952 Nobel Prize in Chemistry for their invention of partition chromatography. By the late 1950s, thin layer, column, and gas-liquid chromatography became the main techniques in the separation and isolation of organic compounds. This was a turning point for Frank's research, which led his team to the cutting edge of fatty acid research.

From the mid-1960 onwards, Frank and his researchers explored the limits of silver ion thin layer chromatography and gas-liquid chromatography (GLC) carried out on 50 m long capillary columns on closely related synthetic isomers (*viz.* positional isomers mono- and di-unsaturated) of C18 fatty acid esters. The publication of his results caught the attention of the international lipid community. St. Andrews under Frank's guidance became synonymous for golf and lipid research. He was quick to focus his attention to the study of basic molecular chemistry of fatty acid molecules. By studying the chemical effects of neighboring participating groups involving oxygenated unsaturated fatty acid molecules, he opened up a new frontier for fatty acid research as potential renewable feedstock for industry. He was literally "entertaining" the chemical world with his creative chemical ideas and the interesting lipid molecules with varying functional groups and structural elements (*viz.* carbocyclic, heterocyclic, heteroatomic, *etc.*) he produced. His work filled the much neglected field of fatty acids in Organic Chemistry to a great extent.

When state-of-the-art analytical techniques (high resolution proton and carbon NMR spectroscopy and mass spectrometry) became available in the mid-1970s, Frank and his researchers were there and ready to offer the world the first glimpse of the behavior of fatty acid esters and their derivatives by nuclear magnetic resonance spectroscopy. He was able to identify most of the carbon and proton nuclei contained in the fatty acid molecule from results obtained on a 90 MHz NMR instrument.

Similarly, when high-resolution mass spectrometers became available, his work on the mass spectrometric analyses of position isomers of fatty acids and other derivatives made waves in the interpretation of the fragmentation patterns of organic molecules. Frank published over 350 scientific papers in international peer reviewed journals. He never held back any "bite" from his research but shared his findings with the entire world unreservedly.

THE WRITER

By the end of 1980s, Frank had reached a critical stage in his career. He was approaching the golden age of 65, and according to university policy, it was time for him to .... retire. This was analogous to bringing a bullet train flying on its track at 300 km per hour to a sudden halt.

It was around 1987, when he wrote me on this issue. For a whole year, he and I debated the issue most seriously. In the end, I regretfully declined his invitation for me to join his University in Scotland. In our exchange of letters, I mentioned that I wished I had the financial resources to invite him to come to Hong Kong and join my research group. Together, I wrote, we could move the frontier of fatty acid chemistry further, as I could leave the writing up of the results to him for publication while I concentrated on the bench work with my team. He confided in his next letter that it was "time to say goodbye to bench work" and focus on writing instead—a decision that made lipid chemistry history.

During the next quarter of a century, Frank produced a written legacy of over 30 titles devoted to lipid science. One of his most significant works was *The Lipid Handbook*, co-edited with Fred Padley and John Harwood (1986, 1994, and 2007) that is often referred to as the "bible" of lipid science and technology (see page 34). His *Lipid Glossary* (1992 and 2000) co-edited with Bengt Herslof was another major contribution. In 1992, Frank was invited to become the Chief Editor of *The Oily Press,* which morphed to *Lipid Technology* under Wiley. Frank remained Chief Editor of the journal until his 90th birthday.

> *"I first met Frank more than 35 years ago when looking into the fatty acid composition of what at the time was the little-known speciality oil obtained from the evening primrose plant.... an oilseed Frank and his lab had already been working with for some time. Indeed, at the time, his lab was one of few in the world with experience in the analysis of this and other minor oilseeds. Over the years, he assisted me greatly in the analysis of vegetable and fish oils and concentrates, providing invaluable advice and independent analytical input. If the analytical data came from Frank's lab you knew it would stand up to any scrutiny. Not only was Frank a true giant of Lipid Chemistry, he was also one of the kindest, gentlest, and most helpful of men."*
>
> **— PETER CLOUGH, consultant to Mylnefield Lipid Analysis**

HONORS AND AWARDS

Frank received many international chemical awards for his contribution to lipid science and technology. The American Oil Chemists' Society (AOCS) awarded him the prestigious AOCS Lipid Award (1973), Alton E. Bailey Award (2005), and Stephen Chang Award (2006). He was named an AOCS Fellow in 1999. He was also the recipient of three medals recognizing his great contributions to lipid science: Kaufmann medal*,* International Society for Fat Research (1976); Chevreul medal*,* Societe Francaise pour l'Etude des Lipides (1990); and Normann medal*,* German Society of Fat Research (1998).

A PERSONAL REFLECTION

Our world was blessed to have Frank Gunstone with us for nearly a century. His early works (1952, 1954, published in *J. Chem. Soc.*) may appear "primitive" to modern-day chemists, who have at their disposal analytical instruments such as LC-MS (liquid chromatography-mass spectrometry), GC-MS (gas-liquid chromatography-mass spectrometry), and Tandem-MS, to mention a few. How fortunate they are to have multi-million-dollar high field NMR instruments to "assist" them in deciphering the structures of their compounds. Little perhaps do they realize that Frank Gunstone's pioneering works (1970–1990) on the NMR and MS properties of fatty acid derivatives played an important part in the development of nuclear magnetic resonance spectroscopy and mass spectrometry. Moreover, Frank left behind a most valuable legacy in the form of a large collection of books and manuscripts on lipid chemistry. He filled the void in the chemistry of fatty acids, which was once a "forgotten field of Organic Chemistry". Frank Denby Gunstone (FDG) is regarded by the fats and oils community as the Father of Lipid Chemistry.

To end my tribute to FDG, I quote an excerpt from a letter from Isaac Newton to Robert Hooke (1676): "Does it surprise you, that if I have seen a little further than others, it is by standing upon the shoulders of giants." Our gratitude goes to Frank, from whose shoulders we have a better view of lipid chemistry. My life was blessed when I met Frank and his wife, Eleanor, in 1965, leading to an association which lasted for over half a century.

Marcel S.F. Lie Ken Jie, was a professor of chemistry at The University of Hong Kong, SAR, China, for 47 years. He is now retired and can be contacted at lkj.marcel@gmail.com.

Enhanced purification of biofuels using highly activated bentonite clay

Brian Cooke

- Fats and oils feedstocks for biodiesel and renewable diesel production contain metals, phosphorous, soap, and other impurities that can impact the efficiency of catalysts involved in fuel synthesis reactions.
- Acid-activated calcium bentonite clay is an effective adsorbent for removing such contaminants before they pass to the catalyst bed.
- When used as a pretreatment, it protects the catalyst and increases reaction efficiency.
- Using the adsorbent in the post-treatment of biodiesel ensures that regulatory fuel specifications are achieved.

Derived from fats and oils, biodiesel and renewable diesel are cleaner, more efficient, and more sustainable than traditional fuels. However, their purification process presents unique challenges for producers. The feedstocks of these "greener" fuels contain various contaminants, such as metals, phosphorous, and soap, which interfere with the efficiency of the catalysts that are required for fuel production. These contaminants poison the catalyst, decrease their activity level, and thereby decrease the overall productivity of the plant. While renewable diesel only requires pre-treatment to remove contaminants from the feedstock, biodiesel needs additional post-treatment to clear the finished product of impurities. Whether pre- or post-treatment, an optimal material for contaminant removal is highly activated bentonite, also known as bleaching clay.

CONTAMINANT ADSORPTION IN BIOFUELS

Bleaching clays, also known as adsorbents, are materials that can bind other substances to their surface without undergoing a chemical reaction. To boost the binding power of Ca-Bentonite, it is subjected to a chemical reaction with acid. It increases the surface area drastically and creates active binding sites for impurities. Based on internal analysis, the active surface area before and after acid activation has shown an increase from approximately 60 m2/g to >200 m2/g and its loose bulk density increases roughly from 800 g/ to 400g/l.

The reason that adsorbents attract impurities is due to their high thermodynamic energy, which is lowered after adsorption. A common example of an adsorbent is carbon, which can purify and soften water through ion exchange. The active centers and the enlarged surface offer numerous docking sites for undesired substances, which can be reliably

bound; in other words, adsorbents are optimized to bind undesired substances.

In the case of biodiesel and renewable diesel, highly activated bentonite clay is the adsorbent most often used to remove soluble impurities. The clay allows deep filtration down to one micron or less, resulting in higher quality feedstock. This improves reaction efficiency and protects catalysts during fuel production. Moreover, effective adsorbent filtration with bentonite clay ensures prolonged catalyst life (exact life extension is catalyst dependent) for renewable diesel production while helping to achieve fuel specifications for biodiesel.

THE ORIGINS OF BENTONITE CLAY

Bentonite is derived from volcanic ash. During a volcanic eruption, lava, gas, and air are propelled outwards. After the ash settles, it is carried by wind to valleys and cavities. Rain and rivers transport the ash further under layers of rock and sand, protecting it from erosion. Over eons, the ash deposits are subjected to high pressures and temperatures, transforming it into bentonite (Fig. 1), while the landscape above undergoes massive changes.

ACID-ACTIVATED BENTONITE

The acid-activated calcium bentonite clay is used to treat biofuels—as well as edible and mineral oils. The adsorbent is first activated through a chemical reaction with acid to drastically increase its surface area and pore volume, thus maximizing its active binding sites for impurities and enhancing its adsorption capacity.

Besides the importance of contamination-binding capacity, adsorbents offer an optimal balance between the ability to remove contaminants as well as properties that improve filtration. This selective removal of impurities helps to minimize feedstock losses and avoids process bottlenecks. Other critical factors related to filterability include adsorbent porosity, particle size distribution, and particle shape. Specialized calcium bentonite adsorbents, such as Clariant's TONSIL® RNF range, offer a broad selection of natural and activated bentonite bleaching clays that can be matched with a variety of feedstock types, process designs, and production requirements for optimal performance. These adsorbents are suitable for pre-treatment of feedstocks such as hydrotreated vegetable oils (HVO) and fatty acid methyl esters (FAME), as well as post-treatment of the finished FAME product.

FIG. 1. Bentonite clay

BIODIESEL PRODUCTION

Biodiesel is a methyl ester that is produced via a process called esterification, in which triglyceride from fat or oil feedstock is reacted with methanol in the presence of a catalyst to form a mixture of fatty acids and the by-product glycerin (Fig. 2). Both prior to and after the reaction, filtration is vital for eliminating impurities since a methyl ester is not considered biodiesel until the proper specifications are met.

During pre-treatment filtration, bentonite adsorbent is used to remove soap, phosphorous, and metals that are present in the fat or oil feedstock. After the reaction, the contaminated fatty acid methyl esters (FAME) are separated from glycerin, which is a by-product. Excessive soap can result from the catalyst reaction with the FFA. In addition, water can increase the likelihood of high soap content. When there is excessive soap remaining in the FAME, emulsification can occur, making it difficult to separate water in the final washing step. Thus, using adsorbent can eliminate emulsification.

Insufficient catalyst removal can occur in biodiesel production, which can cause injector deposits and filter plugging or poor glycerin separation. This latter challenge proves to be especially troublesome in the post-reaction phase since it could lead to reversion during the demethylation process and/or off-spec product. High acid value is another difficulty, typically due to inadequate esterification, which may cause oxidation and shorter shelf life of the product, as well as possible fuel system deposits that would affect the fuel pump and filter operations. Inadequate alcohol removal is a further concern that could result in premature injector failure and serious safety concerns. All of these potential hurdles could be avoided or overcome when using the correct bentonite adsorbent.

RENEWABLE DIESEL PRODUCTION

Renewable diesel is a true hydrocarbon just like diesel and meets the ASTM International's standards, such as D6751. However, the molecular structure of renewable diesel differs from biodiesel. Due to these differences at the molecular level, renewable diesel carries less oxygen and shows unlimited miscibility with conventional diesel.

To produce renewable distillate, highly active nickel-based catalysts, such as Clariant's NiSAT® or HYDEX® catalysts, can be used to remove oxygen and nitrogen from natural oils and greases (Fig. 3).

Here, again, pre-treatment is essential for removing metals since they decrease the life of the catalyst. Through their removal and life of the catalyst increase, the producer can lower their maintenance costs and increase the efficiency of the hydrotreatment process. The first step of the production process involves catalytic decarboxylation of organic molecules, which removes carbon dioxide (CO_2) and water (H_2O) as by-products. Next is isomerization, also based on a catalyzed reaction.

In renewable diesel production, catalyst poisoning is mainly caused by phosphatides. Many phospholipids tend to form micelles when they congregate in the cell membranes of fats and oils. This explains why removing phospholipids by acid or water degumming is needed. Subsequently, residual traces of contaminants can be removed using adsorbents. In particular, non-hydratable phospholipids can be cleared using bleaching clay adsorbents that bind the phospholipids to their surface. These measures protect the catalyst and extend its lifetime, thereby ensuring efficient production of renewable diesel.

SUSTAINED INNOVATION FOR BIOFUEL PRODUCERS

As regulations concerning renewable energy grow increasingly stringent around the world, biofuel producers face greater challenges in meeting the required specifications. Besides the complexities of feedstock impurities, biofuel production is also greatly affected by filterability.

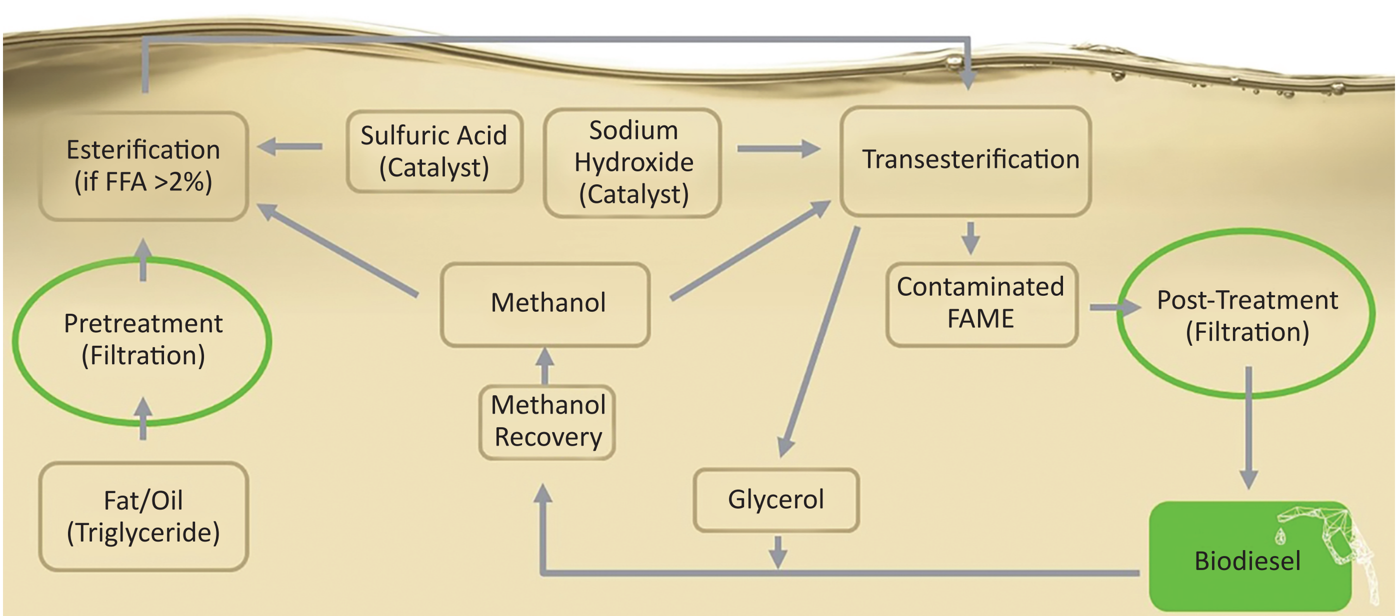

FIG. 2. Biodiesel synthesis with pre- and post-treatment filtration

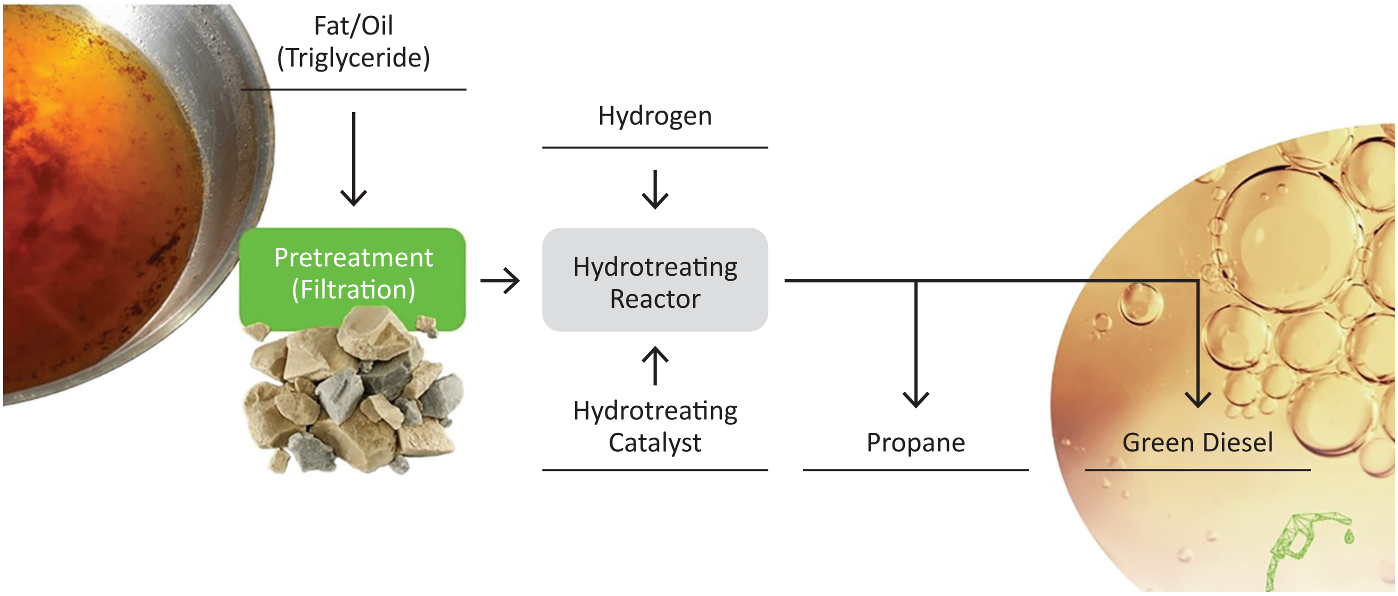

FIG. 3. Production of renewable diesel including pre-treatment

Filtration efficiency also has a major impact on production costs. These difficulties can be mastered through a combination of customized purification strategies and novel adsorbents, like those from Clariant. Moreover, innovative solutions are being continuously developed to help fuel producers enhance their production processes, increase their throughput, improve their plant productivity, and optimize their cost efficiency while fulfilling strict regulatory requirements. To learn more about biofuel purification solutions from Clariant, please visit www.clariant.com/oilpurification.

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Sustainability and pet food

Heather L. Acuff, Amanda N. Dainton, Janak Dhakal, Samuel Kiprotich, and Greg Aldrich

- Life-cycle analysis of pet foods has identified that the most significant impact category to the environment is climate change (quantified as kg co2 eq), with wet foods tending to have a greater impact than dry foods, and dogs having a greater impact than cats.
- Opportunities for improvement in sustainability exist at all phases of the pet food life cycle, including formulation, ingredient selection, manufacturing processes, packaging materials, transportation methods, reduction of food and packaging wastes, and proper disposal of pet waste.
- Following is an abbreviated version of a review article originally published in the May 2021 issue of *Veterinary Clinics of North America: Small Animal Practice 51*: 563–581. Additional tables and references can be found at https://doi.org/10.1016/j.cvsm.2021.01.010.

The overuse of resources has become a concern as world populations increase. The environmental footprint of pet ownership and provision of necessary supplies and food for pets on the use of natural resources, emissions, and waste are also growing. The questions regarding the size of that impact and where opportunities for improvement exist begin with the pet owner and the general public's perception of the topic regarding sustainability and move upstream to the raw material suppliers, food manufacturing companies, packaging producers, and transportation sectors. Overcoming barriers to sustainability will require the implementation of successful intervention strategies, and the pet owner will need to assign value to this effort, as sustainable products are likely to cost more at retail. The following objectives are critical to the discussion of sustainability of pet food: (1) to define sustainability; (2) to describe the life-cycle analysis (LCA) of the pet food industry and identify areas for improvement; (3) to determine how food process, product type, nutrient composition, and ingredient selection might influence the sustainability of pet foods; and (4) to provide information about the pet food LCA in an effort to educate pet owners in areas where they can influence sustainability.

ENVIRONMENTAL IMPACT OF DOG AND CAT OWNERSHIP

According to recent US pet ownership statistics, two-thirds of US households are estimated to own at least 1 pet across nearly 85 million homes. Worldwide, about one-third of households have dogs, and a quarter have cats. Companion animals enrich the lives of towners in numerous ways, such as increasing physical activity, lowering blood pressure, and reducing risks of certain heart diseases. Pet ownership has also been associated with psychological benefits, including increased self-esteem

in children, reduced risk of depression, and increased social engagement and cohesion.

Despite the many rewards of pet ownership, our pet-centric way of life may take a toll on the environment. The growing populations of urbanized pets have been linked to loss of wildlife biodiversity because of predation and disturbance, as well as a greater consumption of goods and services. It is estimated that in the United States alone, cumulative pet industry expenditures reached $95.7 billion in 2019, with pet food and treats making up the largest sales segment (38%).

Several researchers have evaluated the environmental impact of dogs and cats based on annual pet food consumption, with results ranging from 27 to 1444 kg co2 eq per year for dogs, and 43 to 228 kg co2 eq per year for cats. Because pet excrement is a direct product of food intake, it could be argued that pet food production and consumer purchasing behaviors should shoulder the responsibility of environmental stewardship. Thus, considering sustainability as it relates to all aspects of pet food allows for a broader understanding of the environmental impact of our pets.

DEFINING SUSTAINABILITY IN THE PET FOOD INDUSTRY

The US Environmental Protection Agency (EPA) defines sustainability as a harmonious and productive system in which humans and nature could exist, permitting the fulfilment of social, economic, and other requirements of the present generation without jeopardizing the needs and requirements of future generations. From the perspective of pet food production, sustainability has been defined as the ability to produce pet food in adequate amounts while providing the sufficient essential nutrients required to maintain optimum health and viability now and in the future with the smallest environmental footprint. Here, the authors propose a broader definition for sustainability that incorporates the stewardship of companion animals: the conscientious management of resources and waste necessary to meet the physiologic requirements of companion animals without compromising the ability of future generations to meet their environmental, social, or economic needs.

QUANTIFYING CARBON FOOTPRINTS WITH PET FOOD LIFE-CYCLE ANALYSIS

The environmental impact of a food system can be quantified by analyzing all material inputs (energy and natural resources) and outputs (waste and emissions) and their associated costs, a process known as LCA. Following ISO 14044:2006 standards, LCA serves as a globally recognized model framework to study the environmental impact categories associated with a product or process such as climate change (biogenic and land use and transformation), ozone depletion, human toxicity risk (cancerous and noncancerous), particulate matter, ionizing radiation, photochemical ozone formation, acidification, eutrophication (terrestrial, freshwater, and marine), freshwater ecotoxicity, and natural resource use.

The LCA of dog and cat foods is highly complex considering the variety of raw materials, manufacturing technologies, and packaging options that exist today. The environmental impact of food and agricultural systems can differ considerably. Geographic location also influences the environmental burden of agricultural products, in terms of both production and transportation. In addition to raw material extraction,

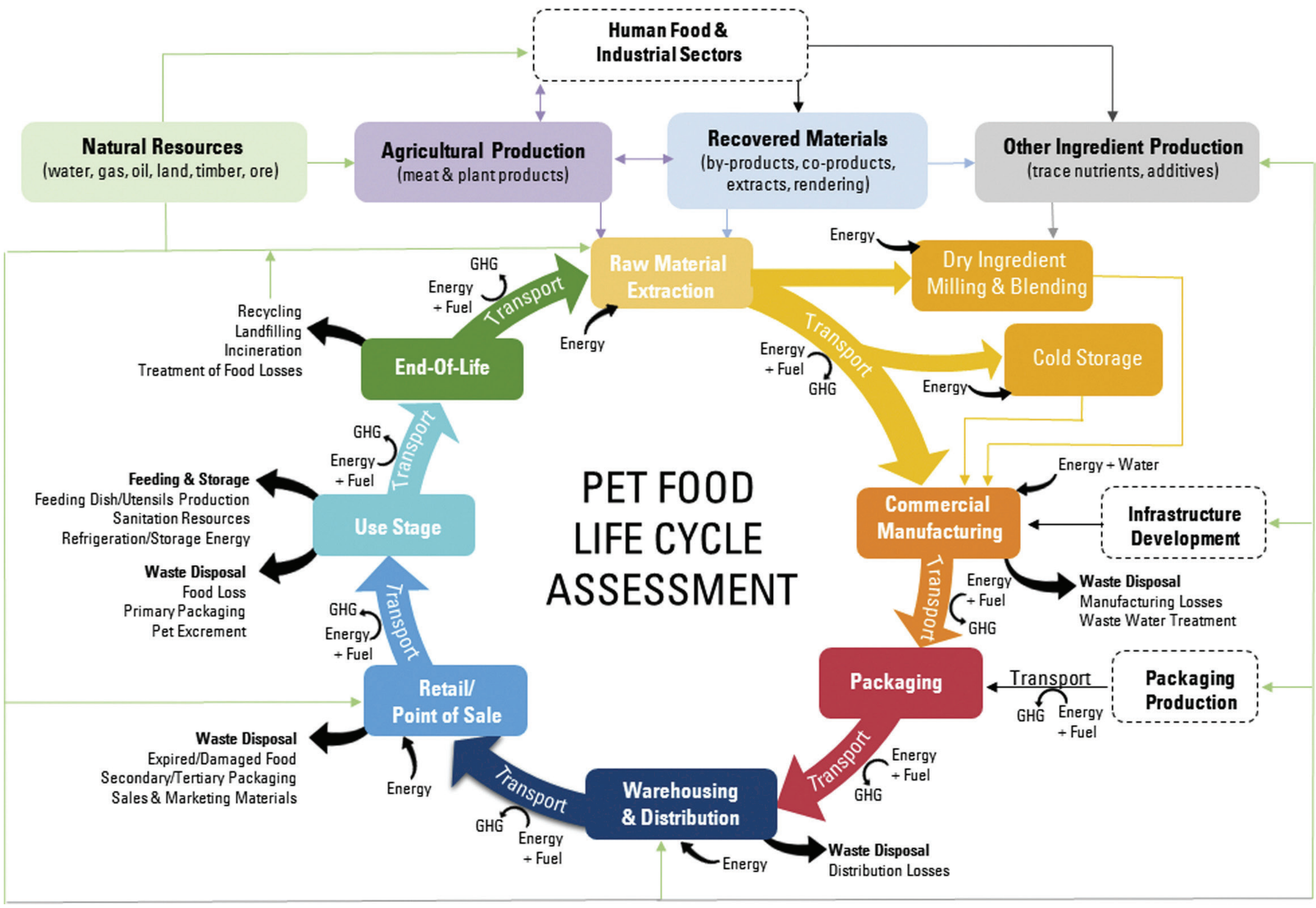

FIG. 1. A generic LCA for commercially prepared pet food beginning with raw material extraction and tracing through manufacturing, packaging, distribution, retail, usage, and end-of-life disposal.

manufacturing technology (eg, extrusion, canning, baking, freeze-drying), nutritional composition of product (eg, moisture and protein level), packaging specifications, distribution channel, and storage and usage requirements are additional factors interlinked with a product's carbon footprint.

Despite these many complexities, in 2018 the European Commission adopted the Product Environmental Footprint Category Rules (PEFCRs) as a standardized model for calculating environmental impacts for the full life cycle of prepared pet foods for dogs and cats. The model development consists of 4 LCA studies of complete pet foods sold in Europe representing cat and dog foods, kibble, and canned foods. Dog food (wet and dry) collectively had a greater environmental impact than cat food because of higher consumption volume of dog food. The estimated impact of wet food also exceeded dry food because of the high use of natural resources for packaging production (tin plating). Overall, the most relevant impact categories for pet food were determined to be climate change, eutrophication (freshwater, marine, terrestrial), land use, and natural resource depletion (water, mineral, and fossil). Although the PEFCRs were developed using data sets for EU energy reporting, pet food production in the United States follows a similar life cycle (Fig. 1), and thus, the principles of the PEFCRs could be applied to the US pet food systems.

DIET SELECTION AND NUTRITIONAL COMPOSITION

There are two defining attributes that influence the path of a pet food product's life cycle. Diet selection, which dictates the intended species, life stage, food format, and inclusion or exclusion of specific ingredients, and nutritional composition, which determines the level of raw materials needed to achieve the desired nutrient levels, both of which have a direct impact on the resources required to construct a product.

Protein is the most expensive and ecologically demanding macronutrient, yet is a key factor for the selection of pet food products by pet owners. Pets require a moderate level of protein in their diets, with Association of American Feed Control Officials minimums set at 18% for adult dogs and 26% for adult cats on a dry matter (DM) basis. However, high-protein formulas (>30% crude protein on a DM basis) are commonly marketed for both species, as more protein may be needed to maintain lean body mass and support the needs of older dogs and cats, and working dogs, as examples. The idea that protein levels in excess of an animal's requirement are beneficial is debatable and adds strain to the increasing global demand for protein for humans, agricultural animals, and companion animals.

There is a belief, shared by 29.4% of dog owners and 21.7% of cat owners, that raw diets are healthier for their pets; however, only 3.9% of veterinary professionals agree with this. One in 5 pet owners also report following raw feeding practices originating from online resources rather than published references or seeking veterinary advice, which may exacerbate nutritional or safety risks associated with raw feeding. With regard to sustainability, raw pet foods are thought to compete with the human food chain because of the high inclusion of edible ingredients. In addition, the handling and storage of the leftover raw pet food can become a safety concern to pet owners because of the high risk of exposure to pathogens. The American Veterinary Medical Association (AVMA) also discourages feeding pets raw animal-based foods, especially those that have not gone through pathogen elimination steps during processing.

RAW MATERIAL SELECTION

Animal-based protein sources

Much of the protein in pet foods originates from animal sources, and there is a trend for increasing both quality and quantity of meat in pet foods. Dog and cat owners generally prefer meat as a source of protein for their pets compared with alternative sources, such as insect proteins, vegetable proteins, or laboratory-grown meats. Animal-based ingredients are considered to be a high-quality source of dietary protein, containing a complete profile of essential amino acids dogs and cats require. However, these tend to have a greater ecological footprint as compared with plant-based proteins (Table 1).

Antibiotic-free protein sources, especially poultry, have become increasingly popular in both human food and pet food. This popularity is attributed to a widely accepted belief that antibiotic-free products are healthier and safer; however, there are no scientific data to support the nutritional superiority of the antibiotic-free animal tissues. Antibiotic-free animal production, in turn, has potentially adverse effects on the sustainability aspects of the food chain because of compromised animal health, reduced production efficiency, and increased costs of production. The AVMA recommends the judicious use of medically important antimicrobials in animal production in order TO sustain their utility for both man and animal.

Animal-based coproducts

Presuming that there will be meat consumption for the foreseeable future, the proper use of all the available resources, including animal by-products, is necessary. Average carcass yield, or dressing percentage, ranges between 50% and 74% of live animal weight for red meat, pork, and poultry products in the United States, leaving behind a significant portion of animal-derived material that does not enter the human food system. When managed responsibly, producers can lessen the environmental effects of organic waste disposal and help recover valuable nutrients. Clean animal offals, for example, provide good-quality protein and higher levels of trace minerals, such as iron, zinc, calcium, and copper, in comparison to muscle tissues and can be incorporated into pet foods in raw, dried, or rendered forms. According to the National Renderers Association, 56 billion pounds of renderable raw material is diverted from landfills and recycled into useable fat, oil, and protein products annually in North America. Rendering also avoids at least 90% of potential GHG emissions when

TABLE 1. Average global warming potential estimates of select insect-, animal-, and plant-origin ingredients with applications in US pet foods

Ingredient	LCA Study Location	Carbon Footprint (kg co2 Eq/kg Functional Unit)
Black soldier fly larvae *a	Germany	1.36–15.1
Plains, ranched beef *b	United States	20.4–23.2
Pasture, finished beef *c	United States	19.2
Feedlot beef *c	United States	14.8
Grassland, grazed lamb *b	New Zealand	19
Hillside, raised lambs *c	England	17.9
Lowland, raised lambs *c	England	10.9
Organic farmed salmon *c	Canada	2.7
Farmed salmon *c	Canada	2.1
Pork *c	United States	2.01–3.02
Chicken *c	United States	1.99
Poultry by-product meal	Portugal	0.73
Poultry fat	Portugal	0.67
Hydrolyzed feather meal	Portugal	0.60
Rendered animal protein	United Kingdom	0.15
Rendered animal fat	United Kingdom	−0.77 to 0.15
Rice	United States	1.41–1.88
Potato	France	0.10–0.11
Sorghum	United States	0.60–1.24
Wheat	United States	0.45–1.32
Soybean	United States	0.34–0.70
Oats	France	0.31
Corn	United States	0.30–1.68
Spring peas	France	0.29
Rainfed legumes	Spain	0.23

*a. Functional unit = 1 kg insect protein meal;
*b. Functional unit = 1 kg carcass weight; *c. Functional unit = 1 kg live weight.

TABLE 2. Proximate composition (as-is basis) of select pet food ingredients and coproducts

Ingredient	DM, %	CP, %	Fat, %	TDF, %	Ash, %
Beef, MSM	40.6	15.0	23.5	0.0	2.1
Beef liver	31.0	20.0	3.9	0.0	1.3
Beef heart	24.4	17.1	3.8	0.0	1.0
Beef kidney	23.0	16.6	3.1	0.0	1.1
Beef tripe	18.6	14.6	4.0	0.0	0.4
Animal fat	99.0	0.0	98.0	NR	NR
Meat and bone meal	92.0	45.0	8.5	NR	37.0
Chicken, whole carcass	33.9	18.5	12.0	NR	NR
Chicken, meat and skin	38.2	17.6	20.3	0.0	1.0
Chicken gizzard	23.8	18.2	4.2	0.0	0.9
Chicken liver	26.4	18.0	3.9	0.0	1.2
Poultry fat	99.0	0.0	98.0	NR	NR
Chicken meal	95.9	64.2	12.2	NR	14.7
Poultry by-product meal	93.5	59.0	13.5	NR	16.0
Feather meal	93.0	85.0	4.0	NR	3.9
Dried whole egg	96.6	47.2	41.1	0.0	3.6
Eggshell meal	99.6	6.6	0.0	NR	53.6
SD egg white	91.1	76.0	0.1	NR	4.9
SD inedible whole egg	93.5	45.8	34.9	NR	3.9
Corn meal, whole kernel	89.7	8.1	3.6	7.3	1.1
Corn starch	91.7	0.3	0.1	0.9	0.1
Corn germ meal	90.1	28.4	6.0	45.0	3.9
Corn gluten meal	89.1	55.2	1.1	NR	1.2
Corn protein concentrate	91.4	72.1	2.3	NR	0.8
Corn fiber	98.9	11.0	6.0	71.4	0.9
DDGS	90.2	26.8	9.0	NR	4.7
Soybean flour, full fat	96.2	38.1	21.9	NR	5.9
Soybean meal, expeller	89.0	42.0	3.5	NR	6.0
Soy protein isolate	95.0	84.6	0.6	NR	3.8
Soybean hulls	90.9	12.6	2.4	NR	4.4
Wheat flour, whole grain	89.7	13.7	1.9	12.2	1.6
Wheat flour, white	88.1	10.3	1.0	2.7	0.5
Wheat germ meal	89.0	25.0	7.0	NR	5.3
Wheat gluten	93.3	75.6	0.8	NR	0.8
Wheat middlings	89.5	16.6	4.0	NR	4.5
Wheat bran	89.0	14.8	4.0	NR	6.4

Abbreviations: MSM, mechanically separated meat; NR, not reported; SD, spray-dried; TDF, total dietary fiber.

compared with industrial composting, which is equivalent to removing more than 12 million cars from the road.

PLANT-BASED INGREDIENTS

Exchanging protein sources of animal origin with those of plant origin has been proposed to improve the sustainability of pet foods by using fewer natural resources and maintaining a smaller carbon footprint. Animal-based proteins are widely perceived as superior in quality for dogs and cats compared with plant-based proteins; however, the relative digestibility has been reported to be similar between both sources. Plant-based proteins generally contain a limited amount of 1 or 2 essential amino acids, which reduces their overall protein quality. However, by combining complementary ingredients, those that provide an abundance of the limiting amino acids of the other, the overall quality of plant-based protein can be at least as good as that from animal-source proteins. Dogs, being omnivores, are well adapted for a plant-based diet; however, cats are obligate carnivores, so are not able to meet their nutritional requirements from unsupplemented plant-based diets alone.

In addition to providing bioavailable protein, fat, and energy to pets (Table 2), plant-based ingredients and their coproducts possess food-functional properties as well. An ingredient that is currently underutilized but has substantial availability includes distillers dried grains with solubles (DDGS) derived from ethanol production. For instance, 50 kg of corn yields approximately 20.8 L of ethanol, which reduces the dependence on fossil fuels and generates 13.9 kg of DDGS. DDGS contain moderate levels of protein and fermentable fiber and improve palatability in pet food applications. Plant-based coproduct inclusion in foods for pets supports environmental sustainability by using every aspect of the respective crop and supports economic sustainability by increasing the number of competitively priced ingredients available to pet food formulators.

Meat analogues are emerging sources of dietary protein that imitate the texture, appearance, or flavor of animal muscle tissues. Dried texturized vegetable protein is an example of a modern meat analogue that can be made from extruded defatted soy meal, soy protein concentrates, or wheat gluten. Plant-based proteins with elastic or spongy textures, such as wheat gluten and soy protein, also offer versatility in structural formation, and texturized soy proteins can produce meatlike textural attributes with high nutritional quality. These components have been used with success in canned, frozen, or dried pet foods.

Alternative ingredients

Alternative ingredients, such as single-cell organisms (SCO: yeast, fungi, and algae) and insects, are being evaluated as potential meat or plant substitutes. The idea behind use of SCO and insects is that they can be grown on carbon sources that might otherwise be considered unrecoverable in the food production system. For example, a recent LCA of microbial protein produced using a potato wastewater system reported an 87% lower impact on the ecosystem compared with traditional soybean meal production. Microbial proteins are currently being used as a source of high-quality protein and essential fatty acids in aquaculture and are reported to contain higher levels of crude protein compared with conventional animal or plant sources. Insects, such as black soldier fly (Hermetia illucens) larvae, housefly (Musca domestica), and mealworm (Tenebrio molitor), are a major protein source in many countries in Asia, Africa, and Latin America, but are less common in the United States because of negative public perceptions. Application of insect protein as a key ingredient in pet food formulation has gained interest; however, there are few data regarding nutritional quality, and regulatory approvals are pending.

COMMERCIAL MANUFACTURING

The greatest potential for sustainability improvement within the commercial manufacturing sectors are cropland, energy, and water usage. Total annual production of dog and cat food in the United States is estimated to be 9.8 million metric tons. Through LCA, the environmental impact translates to roughly 851 gha of cropland, 14 TJ of energy, and 686,821 KL of water used to produce 1 metric ton of pet food. There is room for improvement, but the impacts made by producing food for dogs and cats are estimated to be lower than many human food product industries.

Impacts on cropland are not directly affected by processing, but energy usage and water could be decreased with operational planning, such as installing more energy efficient equipment or reducing the amount of water used during extrusion or retort processing. A tuna canning plant for pet food in Thailand reduced their water consumption by 32% by switching to hot water and reducing water usage when cleaning cans, cooling cans with pressurized spray nozzles, and teaching employees about the importance of using less water and how they could make a difference. Many such decisions could be considered when new pet food manufacturing facilities are built.

FOOD PACKAGING

Food packaging serves many important functions, including protecting food from spoilage and nutritional degradation, improving efficiencies in distribution and storage, and serving as a source of information to feed regulators and pet owners. Pet food bags and containers are commonly constructed from layers of plastic (polyethylene and its derivatives), paper and paperboard, or metals (aluminum, tin, or steel). Most pet food packages are also designed for single use and nonrecyclable, leaving pet owners few options besides disposal. Food containers and packaging waste are estimated to make up just under one-third of all municipal solid waste in the United States. Packaging developers face many challenges with regards to sustainability. For sustainable packaging to be effective, it must reduce food waste, preserve food quality, and prevent food contamination. It must also address the issue of plastic waste accumulation in the environment. In addition, the materials must also be nontoxic for humans and animals, and cost-effective for feasibility of use.

The next generation of sustainable food packaging research is focusing on the use of renewable starting materials to develop biodegradable polymeric films. For example, dairy-based films are currently being explored as an alternative to petroleum-based packaging by the Agricultural Research Service. Biopolymers from cornstarch, chitosan, carrot processing waste, cellulose, and other agricultural products also show promise for biodegradable film construction in the effort to reduce plastic wastes accumulation in the environment. However, the cost and performance of ecofriendly and lower-barrier packaging compared with synthetic alternatives may still impede their widespread adoption.

TRANSPORTATION AND DISTRIBUTION

The EPA estimates a total 6677 million metric tons of GHG emissions were produced in the United States in 2018, of which transportation was the largest contributor at 29%, followed by electricity (27%), industry (22%), commercial and residential (13%), and agriculture (10%). The concept of “food miles” is an important consideration because many raw materials, packaging, and finished products embark on global transport through its life cycle. Reduction of pet food’s carbon footprint through sourcing local or regional raw materials is a marketing strategy that has gained popularity.

Many of the early pet food life-cycle phases use bulk transportation of dry ingredients, which minimizes the number of vehicles required, and thus the environmental burden. However, when transportation of high-moisture commodities, such as fresh or frozen animal or plant products, is required, the use of refrigerated trucks can exacerbate energy consumption.

CHALLENGES

There are many aspects of the pet food industry that are sustainable, such as using coproducts from the human food industry and decreasing energy and natural resources used during production. In fact, pet food production is more sustainable than many human food processing industries in terms of cropland, energy, and water usage.

Still, pet food companies respond to the values of pet owners, and negative perceptions of coproducts and novel ingredients, as well as expectations for increasingly rapid product delivery, limit the pet food industry’s ability to adopt some of these practices is limited. Veterinarians, on the other hand, are uniquely positioned to educate pet owners when they bring their animals in for examinations. This education could be in the form of providing more information about the benefits of coproducts discussed here and how to decrease the impact of their pets on the sustainability of pet food. An increase in pet owner awareness and interest in sustainability will encourage the pet food industry to continue improving in this area.

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Hibernating brown bears are protected against atherogenic dyslipidemia

Sylvain Giroud and Isabelle Chery, *et al.*

- After about four months of hibernation, brown bears display a typical plasma lipid profile of a phase 2 fast with lipolysis that provides the main source of energy during winter.
- In humans, this would lead to substantial decreases in triacylglycerol and cholesterol levels, along with increased lipid concentrations in both low-density lipoprotein and high-density lipoprotein particles, as well as protein and muscle impairments.
- In contrast, hibernating bears appear able to manage large fluxes of triacylglycerol and cholesterol without the classical adverse effects associated with prolonged fasting in humans and non-hibernators.
- Following is an abbreviated version of an article originally published in *Scientific Reports 11*: 18723, September 2021, that sheds light on this incredible adaptation. Complete figures and references can be found at https://www.nature.com/articles/s41598-021-98085-7.

Mammalian hibernation is a seasonal adaptation that allows individuals to survive harsh environmental conditions and food shortage during winter. During the active season prior to winter, hibernating mammals accumulate large fat reserves and nearly double their body (fat) mass from spring to early fall, which would be considered as an obese condition in humans. Then, hibernating mammals enter a state of depressed metabolism, known as torpor, which leads to substantial reduction of energy needs and enables individuals to survive the winter.

In small (< 8 kg) hibernating species, hibernation corresponds to successive multi-day or -week torpor bouts, during which individual metabolic rate (MR) is reduced on average by ~ 95% of basal rates with body temperature lowered below 10°C. These torpor bouts are interspaced by euthermic phases lasting only few hours. However, some mammalian hibernators of medium (10–20 kg) or large body size (> 20 kg) or living in tropical and subtropical areas do not periodically rewarm during winter, so the torpid state corresponds to their entire hibernation.

Bears hibernate at moderate hypothermia (30–36°C) while lowering their MR by 75% of basal rates during winter. Bears and most other hibernating mammals, or "fat-storing", hibernators do not feed during several months in winter and primarily fuel their energy needs via oxidation of lipids, notably saturated fatty acids (SFAs), mobilized from the white adipose tissue.

Fat-storing hibernating mammals show marked seasonal changes of lipid metabolism, with drastic increases of all lipid levels during hibernation compared to the summer

active period. Increased levels of 1.6 to twofold of non-esterified fatty acids (NEFAs), triacylglycerols (TAGs), and total cholesterol (CHT) were reported in hibernating marmots (*Marmota flaviventris*), golden-mantled ground squirrels (*Callospermophilus lateralis*), thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), and European badgers (*Meles meles*) during winter.

Recently, we demonstrated that a large hibernator, the brown bear, shows seasonal shifts in its lipid profile resembling those in small hibernators. Also, denning American black bears (*Ursus americanus*) showed a significant doubling of NEFA levels in all classes (saturates, monemes and polyenes), along with a 33% increase in albumin, *i.e.,* the plasma fatty acid binding protein, leading to higher NEFA/albumin ratios (4:1) compared to those (3:1) of active black bears in summer. However, despite such seasonal hyperlipidemia and hypercholesterolemia, hibernators do not spontaneously develop pathophysiological syndromes, such as atherosclerosis or other complications linked to lipid peroxidation and oxidative damages during hibernation.

The effect of several months of hibernation on plasma lipid levels resembles that occurring during several days of fast (phase 2 of prolonged fasting) in non-hibernating mammals, such as rodents, rabbits, and humans. However, when subjected to prolonged fasting, rodents and humans show, substantial decreases in TAG and CHT levels, along with increased lipid concentrations in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles, as well as protein and muscle impairments.

In humans, dysfunction of lipoprotein metabolism is associated with hyperlipidemia and hypercholesterolemia, which are direct causes for the development of atheroma plates and ultimately thrombosis. Moreover, excess lipids, such as cholesterol, are associated with the generation of intimal oxidative stress and inflammatory processes, leading to necrosis, fibrosis, and calcification. In that process, oxysterols, which are oxidized derivatives of cholesterol, and isoprostanes, such as prostaglandin F2 isomers, are known for their pro-inflammatory and pro-oxidative properties and these molecules play key roles in the process of atherogenic dyslipidemia.

Instead, hibernators seasonally increase levels of lipoproteins, *e.g.,* very low-density lipoprotein (VLDL) and LDL, responsible for the transport of lipids from the liver to peripheral tissues, during winter, as observed in hibernating golden-mantled ground squirrels and thirteen-lined ground squirrels.

Further, particle size of HDL, involved in the delivery of excess lipids including cholesterol from peripheral tissues to the liver for excretion, increase without any signs of pathological effects in hibernating thirteen-lined ground squirrels. Also, hibernating golden-mantled ground squirrels exhibit significant higher plasma CHT concentration per HDL-cholesterol particles than individuals during the pre-hibernation period. Serum cholesterol and PL (at the exception of phospholipid-ethanolamine) increase in hibernating *vs.* summer active black bears, and variation as large as 650% in total plasma cholesterol along with major changes in lipoproteins occur between early-winter and early-spring, in the European badger.

Although excess and undesired lipid molecules, including cholesterol, are usually lost from the body through fecal excretion or via conversion into bile acids, lipids can also be re-esterified via a futile cycle between HDL and VLDL/LDL within the organism. To date, most of the studies on lipid trafficking and lipoprotein dynamics during hibernation have been conducted in small hibernators, which typically rewarm and reverse metabolism at regular intervals during hibernation. However, little is known to that respect in large species, such as bears, which do not eat, drink, urinate, defecate or exhibit arousal episodes, hence cannot eliminate excess cholesterol, during hibernation. Therefore, one would expect unique physiological and biochemical adjustments of cholesterol and lipoprotein metabolisms in hibernating bears during winter.

Our study investigated the lipoprotein and cholesterol metabolisms in free-ranging Scandinavian brown bears captured both in the summer active season and during their six to seven months of hibernation. We performed analyses of lipoprotein composition and assessed activities of key enzymes of lipid metabolism in blood plasma (to assess lipid trafficking and cycles) and determined lipid composition in skeletal muscles (reflecting cholesterol utilization). We further measured the levels of oxysterols and isoprostanes (prostaglandin F2 isomers).

Hibernating brown bears selectively retain unsaturated fatty acids, which are more prone to peroxidation than saturated lipids with less unsaturation, in their tissues during winter. Hence, we also determined plasma antioxidant capacities and reserves along with oxidative damages in plasma and muscle to determine possible oxidative implications linked to changes in lipid and lipoprotein metabolisms during hibernation. We hypothesized that bears manage the large fluxes of lipids and sterols during their hibernation by increasing lipoprotein lipid concentrations, allowing the activation of futile cycles of re-esterification via lipoprotein metabolism.

Fourteen bears, including two animals studied during two consecutive years, were included in this study. During winter, bears hibernate at moderate (30–36°C) hypothermia, and the torpid state hence corresponds to their entire hibernation from November to April.

PLASMA AND MUSCLE LIPID LEVELS

Plasma concentrations of the main lipid categories and molecules were higher in hibernating brown bears than summer-active individuals. Specifically, we found significant higher plasma levels of CHNEs (53% increase), CHT (42% increase), NEFAs (67% increase), PLs (24% increase), and TAGs (twofold increase) during hibernation (Fig. 1A). In addition, plasma concentration of ßOHB was tremendously higher, by 120-fold, in brown bears during winter hibernation than the summer active season (Fig. 1B).

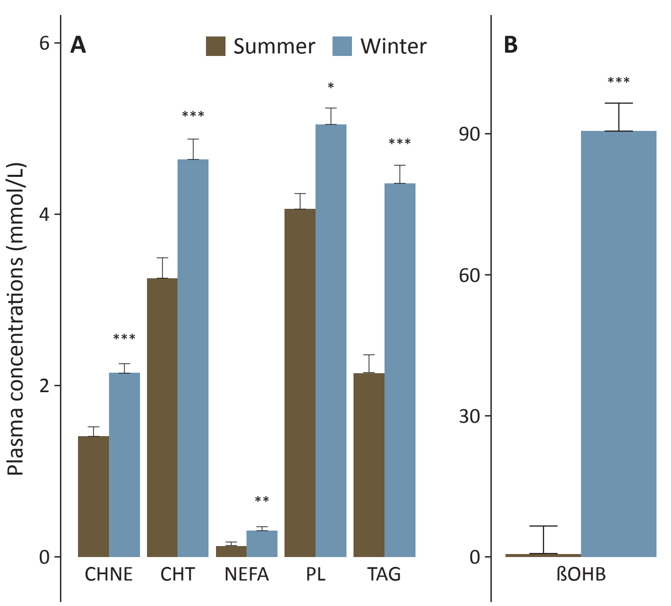

FIG. 1. Plasma concentrations of (A) the main categories of lipids and (B) ß-hydroxybutyrate (ßOHB) from summer active (summer) and winter hibernating (winter) brown bears. In contrast to summer active individuals, winter hibernating bears were all torpid as indicated by their body temperature during hibernation. The lipid main categories are non-esterified cholesterol (CHNE), total cholesterol (CHT), non-esterified fatty acids (NEFA), phospholipids (PL), and triacylglycerols (TAG). Error bars represent standard errors. Winter levels differing significantly from their respective summer level are denoted by subscripts (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

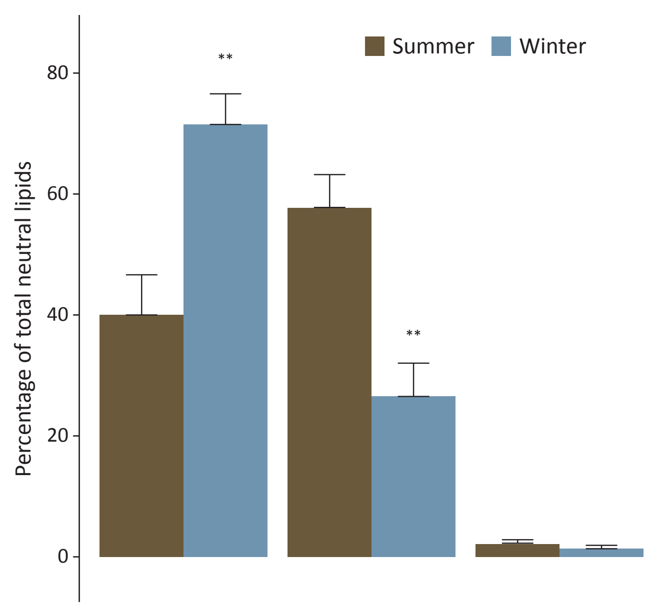

FIG. 2. Muscle levels of neutral lipids according to season. Proportions—% of total neutral lipids—of triacylglycerols (TAG), total cholesterol (CHT), and non-esterified cholesterol (CHNE) in skeletal muscle of summer active (summer) and winter hibernating (winter) brown bears. In contrast to summer active individuals, winter hibernating bears were all torpid as indicated by their body temperature during hibernation (see Table 1). Error bars represent standard errors. Winter levels differing significantly from their respective summer level are denoted by a subscript ($p < 0.01$).**

Muscle concentrations of neutral lipids were also different in brown bears during winter hibernation compared to the active state in summer. Hibernating bear muscles showed a 77% higher level of TAGs and a 2.1-fold lower level of CHT compared to muscles from summer active individuals. No significant seasonal change was detected in the level of CHNEs in muscle (Fig. 2).

LIPOPROTEIN COMPOSITION, SUBCLASSES AND SIZES

Composition of lipoproteins was substantially different in brown bears during winter hibernation compared to the summer active phenotype. We found significant increases of TAG levels by 9, 1.5, 3.2, and 1.4-fold within all lipoprotein classes, namely VLDL, LDL, IDL, and HDL respectively, from winter hibernating bears compared to summer active animals. Further, concentrations of CEs, CHF, CHT, and PLs increased by 8.8, 1.5, 2.8-fold on average in VLDL, LDL, and IDL respectively, but no significant changes were detected in the HDL subunit between winter hibernating and summer active bears).

Similar to lipoprotein composition, subclass proportions and sizes of lipoproteins were also altered, although in different ways between classes/sizes, in hibernating brown bears during winter compared to active individuals in summer. Plasma proportions of all subclasses of HDL decreased significantly by 20%, except HDL2b, which is known to be cardioprotective, that increased by 40% in hibernating bears versus summer active animals. Further, we observed a higher plasma proportion of large-size HDL particles, *i.e.,* those with a diameter greater than 12.9 nm, in brown bears during winter hibernation than during the summer active. No significant seasonal change was detected in the ratio between LDL and HDL (LDL/HDL).

ENZYMATIC ACTIVITY LEVELS

Activities of enzymes involved in lipid and lipoprotein metabolisms were substantially modified in hibernating bears during winter compared to active individuals in summer. We found a significant 36% increase in the activity of plasma CETP and a significant 40% reduction of the activity of plasma LCAT in hibernating brown bears versus summer active animals. No significant seasonal change was detected for the activity of PLTP.

OXYSTEROLS AND ISOPROSTANES

Among the different oxysterols and isoprostanes for which MRM assays were developed, 7ß-hydroxycholesterol, 8-iso PGF2α, 8-iso-15(R) PGF2α, and 15(R)-PGF2α remained below the limit of detection in all samples. The limit of detection was also not reached for 7-ketocholesterol in 7 of 16 winter samples, and for 7α-hydroxycholesterol in 8 of 16 summer samples and in all winter samples but one. On the other hand, 11ß-PGF2α was nicely detected in all samples.

We found significantly lower plasma concentrations of both the oxysterol, 7-ketocholesterol, and the isoprostane, 11ß-PGF2α, in hibernating brown bears during winter compared to active individuals in summer.Plasma levels of 7-ketocholesterol and 11ß-PGF2α correlated negatively with HDL 12-9 nm and positively with proportions of HDL3c small particles across all individual brown bears. Further, the level of 11ß-PGF2α was negatively associated with proportions of HDL2b, known to be cardioprotective, but not with that of 7-ketocholesterol.

BLOOD ANTIOXIDANT DEFENSES AND OXIDATIVE STRESS MARKERS

The overall antioxidant capacity of blood was altered according to the seasonal status of the brown bear. We found a significant 20% greater half-time of hemolysis (HT50; KRL test), *i.e.,* a higher antiradical resistance of red blood cells during winter hibernation. Interestingly, RESEDA-2, the second contingent of antiradical defense reserves corresponding to sulfatases, showed a significant increase of 27%, which was paralleled by a 40% reduction of RESEDA-3, composed of glucuronidases, in winter hibernating bears compared to summer individuals. We observed no significant seasonal difference in RESEDA-1, *i.e.,* glucosidases, between seasons.

We found that antiradical resistance of red blood cells (half-time of hemolysis of the KRL) was negatively correlated with a marker of lipid peroxidation, *i.e.,* malondialdehyde (MDA)-protein adducts, but was not significantly associated with a marker of protein oxidation, i.e., protein carbonyls. Muscle level of MDA-protein adducts was significantly lower in brown bears during winter hibernation *vs.* summer active season. Further, we observed a trend to lower level of protein carbonyls in hibernating bears compared to summer individuals. In contrast, plasma level of MDA-protein adducts, but not that of protein carbonyls, was significantly higher by 30% in winter hibernating brown bears compared to summer active individuals.

These findings highlight the unique ability of a large hibernator, the brown bear, which does not excrete excess cholesterol (by defecating and urinating) during hibernation, to challenge the handling of large fluxes of lipids while fasting during several months in winter. During hibernation, their plasma lipid profile is typical of a phase 2 fast with lipolysis providing the main source of energy during winter. Such an increase in lipid mobilization reflects the occurrence of a switch in substrate metabolism toward the sparing of muscle proteins during hibernation.

Our results further indicate that hibernating bears handle fluxes of TAGs and CHT via futile cycles and re-esterification through lipoprotein metabolism. Specifically, the lipid composition of HDL particles remains stable, while solely HDL2b subunit, known to be cardioprotective, increases during hibernation. In addition, hibernating bears increase blood antioxidant capacities and selectively mobilize SFAs, which are less prone to peroxidation than unsaturated fatty acids, to limit oxidative damages associated to higher lipid fluxes (Fig. 3, page 32).

Together, these results show that after approximately four months of hibernation, brown bears display a typical plasma lipid profile of a phase 2 fast with lipolysis that provides the

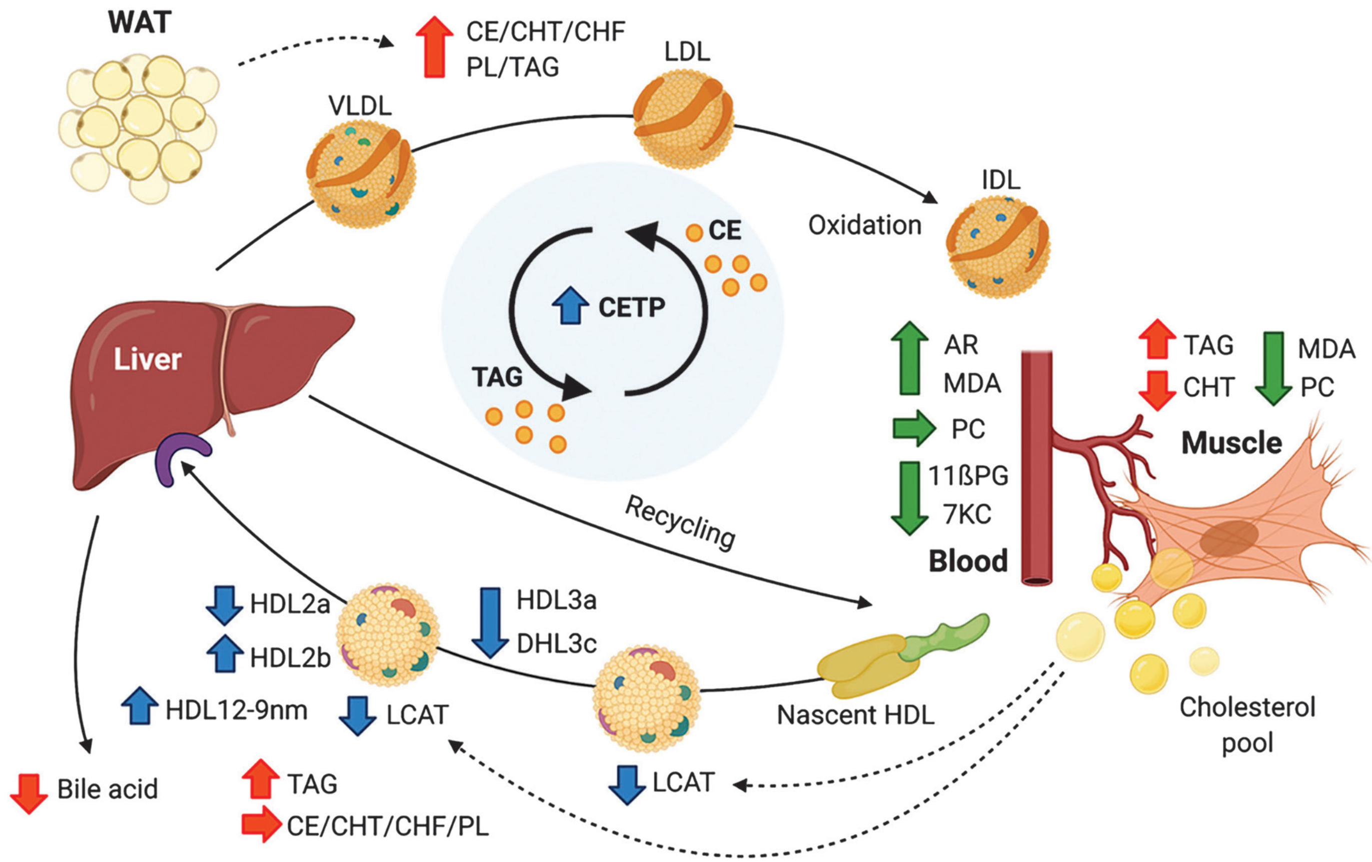

FIG. 3. Futile cycles and re-esterification of lipids in hibernating brown bears during winter. Lipids are released from the white adipose tissue (WAT) and mobilized through the bloodstream for oxidation at peripheral tissues. The increased enzymatic activity of the cholesteryl ester transfer protein (CETP) in the plasma allows hibernating bears to recycle cholesterol via exchanges of cholesteryl-esters (CE) and triacylglycerols (TAG) between lipoproteins. CE is transferred from high-density lipoproteins (HDL) to very low- (VLDL), low- (LDL), and intermediate-density lipoproteins (IDL), which fuel peripheral tissues, e.g., skeletal muscle. Further, a reduced rate of cholesterol synthesis at peripheral tissues, along with a lower activity level of the lipoprotein-bound lecithin-cholesterol acyltransferase (LCAT), lead to maintain constant cholesterol contents in HDL, while increasing HDL size and reducing numbers of HDL particles 2a (HDL2a), 3a (HDL3a), and 3c (HDL3c) except for HDL subunits 2b (HDL2b), which are known to be cardioprotective and increase during hibernation. Also, the pro-inflammatory and pro-oxidative properties of the blood is reduced in winter hibernating brown bears as indicated by lowered plasma levels of the oxysterol, 7-Ketocholesterol (7KC), and the isoprostane, 11ß-Prostaglandin F2α (11ßPG), both indexes for atherosclerosis and cardiovascular risks. Because of the unique fast of hibernating bears, excretion of excess cholesterol through bile acid is not possible, leading to increased level of total (CHT) and free cholesterol (CHF), CE, TAG and phospholipids (PL) in plasma, but not of muscle CHT level which decreases. Along with these higher lipid fluxes, blood antiradical resistance (AR) is increased, dampening and/or reducing oxidative damages linked to lipid auto-oxidation, e.g., MDA-protein adducts (MDA) or protein-carbonyls (PC), in plasma and peripheral tissues, including skeletal muscles. Arrows indicate the directions of the changes. Colors of the arrows represent different components concerned by the changes: red for the lipid categories, blue for enzymatic activities and lipoproteins, and green for the players involved in inflammatory and pro-oxidative processes. Created with https://biorender.com.

main source of energy during winter. As described in studies of fasting, lipolysis provides many times more energy than required to sustain life, leading to high contents of all lipid categories in low- and medium-density lipoproteins. Our results suggest that hibernating bears handle fluxes of TAGs and cholesterol via futile cycles and re-esterification through lipoprotein metabolism, keeping the lipid composition of HDL particles stable, while increasing the HDL2b subunit, which is known to be cardioprotective. Despite higher lipid fluxes, oxidative damages seem to be limited, if not reduced, due to greater antioxidant capacities of the blood and the selective mobilization of SFAs, which are less prone to peroxidation than unsaturated fatty acids. Hence, hibernating bears appear able to manage large fluxes of TAG and cholesterol without the classical adverse effects associated with prolonged fasting in humans and non-hibernators.

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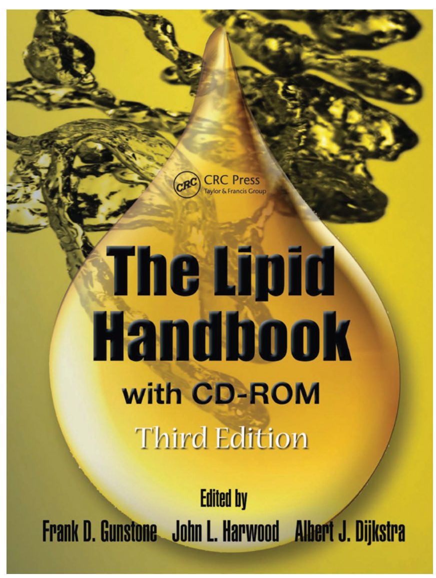
An essential contribution

Olio is an Inform *column that highlights research, issues, trends, and technologies of interest to the oils and fats community.*

Rebecca Guenard

The first addition of *The Lipid Handbook* was published in 1986. The brainchild of Frank Gunstone, the text serves as both a reference to established research, as well as an introduction to new technologies. Gunstone passed away earlier this year (see tribute on page 12), but the handbook he bequeathed to those of us associated with lipid chemistry is an invaluable legacy.

As tomes go, this one is substantial. Weighing nearly five pounds, it is not just physically impressive; some scientists deem it intellectually imperative for anyone working in a seed oils field.



"I first experienced the book when I started working at Cargill. I thought, 'Holy cow, someone took the time to put this together,'" says Bryan Yeh, who is now president and CEO of American Biodiesel Community Fuels based in Walnut Creek, California, USA. "That book is definitely a constant source of information that you want to have around, because there is always something in there that you can use."

The idea for the handbook was born out of Gunstone's collaborative nature and drive to share knowledge. "Frank approached me about publishing a wide-reaching book that covered everything important about lipids," says John Harwood, biochemist at Cardiff University in Wales, United Kingdom. Between the two of them, they had the knowledge to cover the lipid chemistry and biochemistry, but needed an editor for the industry section. For the first two editions that role was filled by Fred Padley, who worked for Unilever at Colworth House, Sharnbrook, U.K.

By the time the third edition was published in 2007, Fred had retired and they needed another author who could cover the industrial sections of the book. Harwood says Frank recruited Albert Dijkstra, who was R&D director at Imperial Chemical Industries in Izegem, Belgium, at the time.

According to Yeh, one thing that makes *The Lipid Handbook* unique is its thoroughness. Besides some basic calculations, he says, most books are not in-depth enough to be valuable to young engineers who want to understand important industrial applications. Most survey books present content at such a low level that a young scientist needs to seek more references to truly understand. "*The Lipid Handbook* does a good job of exploring topics at a deep level Yeh says. "In cases, where the level is not that deep, it is usually because the information is still nascent."

Harwood attributes the book's comprehensiveness to the curiosity all its editors have for budding areas of lipid research. For example, besides researching the role of lipids in human disease, he has studied algae as a sustainable source of lipids. Each editor piggybacks their interest in bourgeoning research upon extensive knowledge in their focus area. "Frank had quite a wide knowledge of the chemistry," says Harwood. "He was publishing new textbooks and collaborating with many different people. He was aware of a lot of advancing areas of lipid chemistry."

Gunstone's chemistry background inspired another notable feature of the book. The dictionary section of the *Lipid Handbook* was modeled after a directory of organic compounds. It describes the physical, chemical, and structural data for 13,000 lipids and related compounds arranged as an alphabetical list.

"The first addition was 571 pages of text and then 314 pages of the dictionary," says Harwood. "It was Frank's idea to call it *The Lipid Handbook*. I always thought that was a bit ironic, since a handbook implies something fairly small. But even the first addition was 890 pages. The third addition is now 791 pages of text and 856 pages of the directory. The combination of the two has been pretty useful to readers."

continued on page 37

What COP26 did and didn't accomplish

Regulatory Review is a regular column featuring updates on regulatory matters concerning oils- and fats-related industries.

Alice C. Hill, CFR Expert, and Madeline Babin

Countries made notable commitments in the Glasgow Climate Pact, but they still fell short of the action needed to keep global warming within manageable levels. This excellent summary was originally published November 15, 2021, in *The Council on Foreign Relations 100*, a weekly digest of the biggest foreign policy stories of the week (https://www.cfr.org/in-brief/cop26-climate-outcomes-successes-failures-glasgow).

WOULD YOU CONSIDER THE TWENTY-SIXTH CONFERENCE OF THE PARTIES (COP26) A SUCCESS?

Yes, but barely. The UN climate summit delivered on its primary goal of keeping alive the Paris Agreement's aim to limit global warming to 1.5°C (2.7°F) above preindustrial levels. Nations agreed on the Glasgow Climate Pact, which states that carbon emissions will have to fall by 45 percent by 2030 to keep alive the 1.5°C goal. But the ultimate success of COP26 depends on the details. The fact that success relies on pledges for future action poses risk of failure. And beyond the concern that pledges might not translate into action, agreements in crucial areas fell short.

WHAT DID COUNTRIES AGREE TO?

Notable provisions in the Glasgow Climate Pact include:

- language supporting a "phase-down of unabated coal power," which is the single biggest source of global temperature rise, a first for a UN climate agreement;
- new rules for trading carbon credits across borders, an issue that had evaded resolution since 2015;
- a call for nations to return in 2022 with new, more ambitious targets to curb emissions; and
- a request for a yearly report summarizing nations' annual commitments to reduce emissions.

Nations shared other important pledges during COP26, including:

- The United States and China, the two largest emitters, agreed to work together on climate despite recent rifts in diplomatic relations.
- Over one hundred nations pledged to cut 30 percent of their emissions of methane—a greenhouse gas that dissipates more quickly than carbon but fuels up to eighty times more heating over a twenty-year period—by 2030.
- More than 130 nations, together possessing 90 percent of the world's forests, agreed to halt and then reverse deforestation by 2030.
- Over 450 financial institutions overseeing $130 trillion in assets promised to align their portfolios with the goal of achieving net-zero emissions by 2050.

WHAT WERE THE FAILURES?

COP26 President Alok Sharma had urged negotiators to "consign coal power to history," but that didn't happen. Despite the historic call in the Glasgow Climate Pact for a "phase-down" in coal power, some coal-reliant countries have indicated that they will not completely stop using coal until the 2040s or later.

Countries also failed to make significant progress on climate finance. The UN Environment Program estimates that developing countries need $70 billion per year for adaptation, and this figure is expected to double by 2030. Going into COP26, poorer nations renewed their calls for financial help from richer nations to adapt to the effects of climate change. They also sought to establish a loss-and-damage fund for developed countries to compensate developing countries for areas irreparably harmed by climate impacts.

But the Glasgow Climate Pact did not resolve the funding challenge. Although the Adaptation Fund, which was established in 2001 to finance adaptation efforts in developing countries, received $356 million in new support at COP26, funding levels remain woefully inadequate. And though the pact presses rich nations to at least double finance for adaptation by 2025, this remains billions of dollars below the projected costs. Wealthier nations also blocked the move to create the loss-and-damage fund. Instead, the pact includes a promise for future dialogue about increased financial support and technical assistance to mitigate climate-related damage.

IS THERE A WAY TO ENSURE THAT COUNTRIES FOLLOW THROUGH ON THEIR PLEDGES?

Accountability remains a central challenge bedeviling global efforts to combat the climate crisis.

The Glasgow Climate Pact includes provisions to increase transparency with the aim of boosting accountability. The pact also urges nations to come back in 2022 with greater ambitions. If implemented properly, the enhanced transparency framework will be an effective tool. And, in 2023, nations are set to meet in the United Arab Emirates to assess progress as part of the Paris Agreement's global stocktake. A well-executed stocktake will evaluate whether nations are fulfilling their commitments and could guide decision-making on new emissions-reduction targets.

WHAT IS THE PROSPECT OF FUTURE PROGRESS THROUGH THE COP PROCESS?

Despite the shortfalls, progress was made, but ensuring that it is sufficient remains a challenge. There are no global courts or mechanisms empowered to enforce these pledges. Progress rests on the weak pillars of goodwill, though peer pressure among world leaders could help.

A lack of women and young people in decision-making on the earth's future has also fueled skepticism about the COP process. But protests led by women, indigenous, and youth activists at the Glasgow conference could provide the push that leaders need.

In closing remarks at the summit, UN Secretary-General Antonio Guterres recognized what he called the "climate action army." Guterres acknowledged the power of activists to propel governments and companies beyond words and into action. He urged them: "Never give up. Never retreat. Keep pushing forward."

Alice Hill is the David M. Rubenstein senior fellow for energy and the environment at the Council on Foreign Relations, where Madeline Babin is a research associate, climate change policy.

OLIO

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"The work that Dr. Gunstone did with this tremendous book was to guide the advancements of lipids in health, nutrition, and medicine," says Doug Bibus, president of Lipid Technologies in Austin, Minnesota, USA. He believes the book's chapter on analytical methods has been crucial to lipids gaining their stature in human health. "If you cannot measure a lipid, it is really difficult to understand it," Bibus says.

Along with the chapter that focuses on general analytical methods, there are chapters that cover lipid physical properties and how to measure them. There is a chapter that follows all the lipid metabolic pathways and gives information about enzymes. The chapters on food production do not just talk about how to process and improve food, but also about the health effects of food on the body.

The second chapter of the handbook includes 100 pages dedicated to all the oils available from different plant sources. Yeh says that until recently, most people just consumed the big three vegetable oils: corn, soybean, and canola. "We did not think about some of the other possibilities that we have as alternatives to the big three. Some of them we probably would not have considered contemplating without this book," he says.

That is the ultimate value of *The Lipid Handbook*, according to Yeh. "Sometimes we scientists think we have to invent solutions when they may already exist," he says. Instead of spending hours on Google, potentially being led in the wrong direction, the handbook has contributed to sparks of creativity throughout his career.

One example of such a time was during the increased interest in pursuing algae as a source of lipids. Yeh says it was like the Wild West during the Gold Rush. "Anytime you could find scum, there was an assumption that there were lipids in there," he says. Anyone who had taken the time to consult the handbook would know there are only certain algae that are worth considering for lipid production. Instead, a considerable amount of investment was lost using the wrong type of algae to make lipid production profitable.

Yeh offers another example of how the book benefitted his career: when he used it as a reference to learn about bacterial and fungal strains that produce lipids. His biotech group realized that by adjusting their genomes, some bacteria can be coaxed into producing a valuable, oil-soluble antioxidant. He says the handbook serves as a platform that enables its reader to gain the wisdom necessary to move projects forward.

"When you think about what made someone pursue this type of research, it often comes down to that thick book that has a plethora of information," says Yeh. "It is the epicenter of the information that you need if you want to work in the area of lipids."

The last five years have seen an explosion of lipids research. Harwood says he has been asked when he will publish a fourth edition. "I would like to try and continue *The Lipid Handbook*," he says. "The real problem is that Frank is not easy to replace." He hopes a younger researcher will take up the mantel. "For people who work in lipids, this is a very important book, and it is because of Frank that it ever came into existence."

Olio is produced by Inform*'s associate editor, Rebecca Guenard. She can be contacted at rebecca.guenard@aocs.org.*

Meet Jonathon Speed

Member Spotlight is a slice of life that helps AOCS members get to know each other on a more personal level.

Jonathon Speed introduces his son Monty (Montague), 4, to the Chessington World of Adventures theme park in the UK.

Fast facts

Name	Jonathon Speed
Joined AOCS	2020
Education	PhD (University of Southampton, Southampton, UK)
Job title	Product and Applications Manager
Employer	Keit Spectrometers (Oxford, Oxfordshire, UK)
Current AOCS role	Session chair, 2022 AOCS Annual Meeting & Expo

PROFESSIONAL

What's a typical day like for you?
I start the day with our 15-minute daily project management meeting. The whole team joins so we all know what's important or urgent that day. I'll check in with the development team (I act as the "Voice of Customer" within Keit) and answer any questions they've got on product development. I oversee the calibration of our instruments, too, so I'll make sure that team has everything they need to build robust and reliable calibrations.

My favorite part of my job is…
The really broad range of applications I get to work with. We deal with lots of applications within the edible oil space, but I also get to work with mining and minerals, biotech, pulp and paper, pharmaceuticals, petrochemicals…you name it, I've probably analyzed it.

Why did you decide to do the work you are doing now?
I took a gamble! I wasn't happy in my previous employment and wanted to get out of the lab and into a more customer-facing role. So, I applied for a "sales engineer" role that quickly evolved into "applications scientist" instead. And I haven't looked back once.

Is there an achievement or contribution you are most proud of? Why?
I once calibrated an instrument in our lab, shipped it halfway around the world to a conference, set it up on the tabletop, and turned it on. It started measuring and worked exactly as expected. That was the first time our team had done that, and I was extremely proud of us all.

What event, person, or life experience has had the most influence on the direction of your life?
When I was young, my family moved to Italy. I didn't realize it at the time, but my father was so good at what he did, his employer created roles for him multiple times just to keep him on the team. I find that inspiring and try to emulate it: being a key contributor to the team as a whole regardless of the actual job/role at the time.

PERSONAL

How do you relax after a hard day of work?
Gardening! We've got a small garden in Oxfordshire. I have crammed it full of so many plants it's a bit of a jungle. But wandering around it with my young son is wonderful. I can teach him about nature, growing fruits and vegetables, and how it's OK to leave a bit of the plot wild to help the "bugs, bees, and birds."

What are some small things that make your day better?
Random photos popping up on my phone of family time with my wife and son—especially those that make you think, "what on Earth were we doing when that was taken?!"

PATENTS

Ceramide-containing capsules, ceramide compositions, and cosmetic compositions thereof

Le Claire, L., *et al.*, L'Oreal, US11007134, May 18, 2021

The ceramide composition includes capsules dispersed in the ceramide composition, the capsules comprising a shell and a core. The shell includes a polycaprolactone, a block copolymer, and a surfactant. The core includes ceramide NP, hydroxypalmitoyl sphinganine, 2-oleamido-1,3-octadecanediol, and a hydrophobic solvent. The core has a weight ratio of the ceramide NP of (i) and the hydroxypalmitoyl sphinganine of (ii) to the 2-oleamido-1,3-octadecanediol of (iii) is from 1:2.5 to 1:5. Additionally, the ceramide composition includes hydroxyacetophenone and hydrophilic solvent.

Oil-containing rubber compositions and related methods

Srinivasan, P., *et al.*, Bridgestone Americas Tire Operations, LLC, US11008448, May 18, 2021

Disclosed herein are rubber compositions comprising bio-oil produced by a recombinant cell. Also disclosed are methods of controlling the variability of fatty acid content in bio-oil containing rubber compositions or tires comprising at least one component incorporating the bio-oil containing rubber composition, and a method of providing a bio-oil-containing tire with a reduced carbon footprint.

Methods of refining a grain oil composition to make one or more grain oil products, and related systems

Lamprecht, B.A., *et al.*, POET Research, Inc., US11008531, May 18, 2021

The present disclosure is related to refining one or more grain oil composition streams (e.g., distillers corn oil or syrup) in a biorefinery to provide one or more refined grain oil products, where each grain oil product has targeted amounts of a free fatty acid component and the fatty acid alkyl ester component.

Phospholipid compositions and their preparation

Myhren, F., *et al.*, Aker BioMarine Antarctic AS, US11020438, June 1, 2021

The invention provides improved processes for extracting and preparing polar lipids (in particular, desirable phospholipids) from krill and other biological sources. The inventors have discovered processes through which it is possible to extract phospholipids to give high phospholipid content and a reduction of undesired components.

Stabilized, pure lithium metal powder and method for producing the same

Wietelmann, U., Albemarle Germany GmbH, US11021797, June 1, 2021

The invention relates to a stabilized lithium metal powder and to a method for producing the same, the stabilized, pure lithium metal powder having been passivated in an organic inert solvent under dispersal conditions with fatty acids or fatty acid esters according to the general formula (I) R--COOR', in which R stands for C.sub.10-C.sub.29 groups and R' for H or C.sub.1-C.sub.8 groups.

Process for producing small droplet emulsions at low pressure

Lang, D.J., *et al.*, Conopco, Inc., US11026869, June 8, 2021

The present invention relates to novel process for making oil-in-water nanoemulsions. The oil phase contains oil selected from the group consisting of triglyceride oil and/or petrolatum and a C.sub.8 to C.sub.16 fatty acid just be added during preparation; and the aqueous phase contains specific N-acyl derivatives of carboxylic amino acid as primary emulsifier.

Process of extracting oil from thin stillage

Jump, J., *et al.*, Novozymes A/S, US11028378, June 8, 2021

A process of recovering oil, comprising (a) converting a starch-containing material into dextrins with an alpha-amylase; (b) saccharifying the dextrins using a carbohydrate source generating enzyme to form a sugar; (c) fermenting the sugar in a fermentation medium into a fermentation product using a fermenting organism; (d) recovering the fermentation product to form a whole stillage; (e) separating the whole stillage into thin stillage and wet cake; (e') optionally concentrating the thin stillage into syrup; (f) recovering oil from the thin stillage and/or optionally the syrup, wherein a protease and a phospholipase are present and/or added during steps (a) to (c). Use of a protease and a phospholipase for increasing oil recovery yields from thin stillage and/or syrup in a fermentation product production process.

Patent information was compiled by Scott Bloomer, a registered US patent agent and Director, Technical Services at AOCS. Contact him at scott.bloomer@aocs.org.

Guidelines for the preparation of figures

Figures are becoming increasingly important in academic publishing. Open Access has contributed to this by reducing limitations on the number of figures allowed in a paper and by eliminating color costs. Within the last several years, PubMed and journal websites have begun to display thumbnails of figures alongside abstracts for all their indexed publications. This means that from the initial search for your paper, your figures are already making an impression.

Readers often assess the figures and images that go with a manuscript before reading through the entire paper. If the figures in a manuscript appear to be low-quality, readers will be less inclined to read past the abstract. Visuals can either elevate or detract from the success of a paper, depending on their quality. Of course, good visuals will have the former effect.

Investing in good figures at the start of your research will also save you time and frustration during the review process. If you do not spend the time to create effective visuals from the start, you will most likely have to spend extra time later to recreate, look for, or correct your figures to be able to publish your work.

The following guidelines and tables provide the basic requirements for submitting your manuscript for peer review at an AOCS journal, as well as the post-acceptance figure requirements.

- **For Peer-Review Submission:** Although we encourage authors to send us the highest-quality figures you can, for peer-review purposes we are happy to accept a wide variety of formats, sizes, and resolutions.
- **For Post-Acceptance:** To enhance the online appearance of your accepted article, the below guidelines below will help you to effectively showcase your research.
- **Important tip!** Creating your figures in one of the preferred file types is better than converting an existing figure later on. If you cannot create one of your figures as an EPS, TIFF, or PNG, send us what you are able to create and we'll do our best to present it effectively.
- **Note to journals published in print:** Print quality will be drastically reduced, possibly impacting readability, if you do not supply your images in the preferred formats and resolutions.

Figure preparation checklist

- Are all figures included in your submission as separate files or in a single PDF/Worddocument/LaTeX suite? **Tip!** Single, original, unconverted files are best.
- Do all figures have an accompanying legend that describes the content and explains any abbreviations or symbols? **Tip!** Include your figure legends as a separate section in yourmain text file.
- Are all figures cited in the main text of your article? **Tip!** Ensure all figures are numbered inthe order in which they appear.
- Are all words or symbols in your figures large enough for easy reading by your audience? **Tip!** Closely follow the preferred resolution guidelines for best presentation.
- Are all figures saved in an acceptable file type? **Tip!** Use the preferred file types for bestimage quality. If in doubt, submit a PDF for initial review.
- Is each individual figure file less than 10 MB? **Tip!** Remove excess white space surroundingfigures for smaller file sizes.
- Were figures created between 80 and 180 mm width? 300 to 600 DPI? **Tip!** Higher qualityfigures are more useful to readers.

A good figure legend succinctly describesthe content and enhances understanding with clear labels. Are all figure files named with their appropriate figure number? **Tip!** Using only figure numbers in the file names ensures correct typesetting.

FOR PEER-REVIEW SUBMISSION

File Types	Preferred	Acceptable
Line art: Line art includes graphs, flowcharts, diagrams, scatter plots, and other text-based figures that are not tables. **Important!** If a figure includes both line art and images, follow the line art guidelines.	EPS PDF	Any standard file type. When in doubt, submit a PDF.
Images: Images include photographs, drawings, imaging system outputs (such as MRIs or ultrasound), and other graphical representations.	TIFF PNG EPS	

FOR PEER-REVIEW SUBMISSION

Resolution	Preferred	Acceptable
Line art: Resolution for line art needs to be higher than for images because each individual line must be more precisely rendered. **Tip!** Larger fonts make for easier reading.	600 dpi	As long as it is legible to reviewers.
Images: Though many web-based images often appear at very low resolutions (72 dpi or lower), readers will only benefit from your research if your images offer hi-resolution detail.	300 dpi	
Image Size	**Preferred**	**Acceptable**
Small: Used for small line art and images that will occupy one-quarter of the page.	80 mm canvas size or Pixel dimensions (width): 1800px minimum	Large enough to support review.
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Complete article files, zipped: Submission systems limit total file size to 500 MBs.	Less than 500 MB total.	EEO limits.
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Legends,and labeling	**Preferred**	**Acceptable**
Figure legends or captions should use Arabic numerals, follow the order in which they appear in the manuscript, and explain any abbreviations or symbols that appear in the figure.	A separate figure legend section in the manuscript, after references.	Anywhere clearly indicating which figure it explains.

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Images: Images include photographs, drawings, imaging system outputs (such as MRIs or ultrasound), and other graphical representations.	TIFF PNG EPS	
Resolution	**Preferred**	**Acceptable**
Line art: Resolution for line art needs to be higher than for images because each individual line must be more precisely rendered. **Tip!** Larger fonts make for easier reading.	600-1000 dpi	Must be legible when viewed as an 80 mm or 1800 pixel width, unmagnified.
Images: Though many web-based images often appear at very low resolutions (72 dpi or lower), readers will only benefit from your research if your images offer hi-resolution detail.	300 dpi	
File Size	**Preferred**	**Acceptable**
Individual files: Individual figures may load slowly or time-out slower systems if they are too large.	Less than 10 MB each.	Large files may slow a reader's experience.
Complete article files, zipped: Submission systems limit total file size to 500 MBs.	Less than 500 MB total.	EEO limits.
File Name	**Preferred**	**Acceptable**
File Naming Convention: to facilitate ease of review, name figure files only with the word "figure" and the appropriate number.	1 figure per file.	All figures in a single PDF, Word document, or asa part of a LaTeX submission.
Legends,and labeling	**Preferred**	**Acceptable**
Figure legends or captions should use Arabic numerals, follow the order in which they appear in the manuscript, and explain any abbreviations or symbols that appear in the figure. **Figure 1.** A good figure legend succinctly describes the content and enhances understanding with clear labels.	A separate figure legend section in the manuscript, after references.	Anywhere clearly indicating which figure it explains.

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H&N Health and Nutrition
PRO Processing
S&D Surfactants and Detergents
BIO Biotechnology
LOQ Lipid Oxidation and Quality
IOP Industrial Oil Products
PCP Protein and Co-Products

Review Articles

BIO IOP PCP Biodiesel production from lignocellulosic biomass using oleaginous microbes: prospects for integrated biofuel production

Chintagunta, A.D., *et al.*, *Front. Microbiol. 12*: 658284, 2021, https://doi.org/10.1186/s13068-021-02009-6.

This paper highlights feedstocks used for biodiesel production, such as vegetable oils, non-edible oils, oleaginous microalgae, fungi, yeast, and bacteria, and describes the steps in biodiesel production from lignocellulosic substrates through pretreatment, saccharification and oleaginous microbe-mediated fermentation, lipid extraction, transesterification, and purification of biodiesel. The paper includes a discussion of the importance of metabolic engineering to biofuels and biorefining as well as a note on integration of liquid biofuels, both of which are significant to the establishment of a circular economy.

EAT H&N Analysis of the effect of recent reformulation strategies on the crystallization behavior of cocoa butter and the structural properties of chocolate

Ewens, H., *et al.*, *Curr. Res.Food Sci. 4*: 105–114, 2021, https://doi.org/10.1016/j.crfs.2021.02.009.

In this review, we analyze how different reformulation strategies affect the crystallization behavior of cocoa butter and, hence, the structural and sensorial properties of chocolate. In particular, this work discusses the effect of: (1) CB replacement with emulsions, hydrogels, oleogels, and oleofoams; (2) CB dilution with limonene or cocoa butter equivalents; (3) replacement or reduction of the amount of sugar and milk in chocolate. We found that there is certainly potential for successful novel alternative chocolate products with controlled crystalline properties; however, further research is still needed to ensure sensory acceptance and reasonable shelf-life of these novel products.

EAT PRO H&N Research advancement and commercialization of microalgae edible oil: a review

Xue, Z., *et al.*, *Crit. Rev. Food Sci. Nutr. 101*: 5763–5774, 2021, https://doi.org/10.1002/jsfa.11390.

This article provides an overview of the progress and future directions in promoting the commercialization of microalgal edible oils, including microalgal triglyceride accumulation, suitable edible oil culture strategies for high nutritional value, metabolic engineering, production, and downstream technologies. The integration of the production process, biosafety, and the economic sustainability of microalgal oil production are analyzed for their critical roles in the commercialization of microalgal edible oil to provide a theoretical and scientific basis for comprehensive development and utilization.

EAT Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: a critical review

Yaashikaa, P.R., *et al.*, Bioresour. Technol. *343*: 126126, 2022, https://doi.org/10.1016/j.biortech.2021.126126.

This paper reviews the methodologies for valorization of agro-industrial wastes and their exploitation for generation of renewable energy products. Management of agro-industrial wastes and products is discussed with an eye toward taking a systemic approach to economic development

IOP PRO Sustainable valorization of algae biomass via thermochemical processing route: an overview

Ayub, H.M.U., *et al.*, *Bioresour. Technol. 344*, Part B, January 2022, https://doi.org/10.1016/j.biortech.2021.126399.

This paper provides a review of the applications of thermochemical conversion techniques for biofuel production from algal biomass, comprising pyrolysis, gasification, liquefaction, and combustion processes. The progress in the thermochemical conversion of algal biomass is summarized, emphasizing the application of pyrolysis for its benefits over other processes. The review also encompasses the challenges and perspectives associated with the valorization of microalgae to biofuels ascertaining the potential opportunities and possibilities of extending the research into this area.

LOQ EAT Valorization of kiwi agricultural waste and industry by-products by recovering bioactive compounds and applications as food additives: a circular economy model

Chamorro, F., *et al.*, *Food Chem. 370*: 131315, 2022, https://doi.org/10.1016/j.foodchem.2021.131315.

The waste groups of Actinidia cultivation include leaves, flowers, stems, and roots, while related food industry by-products are represented by discarded fruits, skin, and seeds. All these matrices are underexploited and could be revalued as a natural source of ingredients for food, cosmetics, or pharmaceuticals. Kiwifruit is high in phenolic compounds, volatile compounds, vitamins, minerals, dietary fiber, and other active compounds. It is especially high in vitamin C and phenolic compounds that possess antioxidant, anti-inflammatory, or antimicrobial activities, among other beneficial health properties. These compounds stand out for their digestive enhancement and prebiotic role. Therefore, agricultural and food industry wastes derived from processing kiwi are regarded as useful matrices for the development of innovative applications in the food (pectins, softeners, milk coagulants, and colorants), cosmetic (ecological pigments), and pharmaceutical industries (fortified, functional, nutraceutical, or prebiotic foods

Original Articles

ANA IOP EAT Assessment of rheological methods to study crystallization of palm oil fractions

Tangsanthatkun, J., *et al.*, *J. Texture Stud. 52*: 169–176, 2020, https://doi.org/10.1111/jtxs.12568.

This study provides an evaluation of the use of rheology to characterize soft, semi-hard, and hard fats in relation to determine the crystallization onset, crystallization behavior, as well as microstructure development using either a plate–plate or a starch pasting cell (SPC). The results from this study demonstrate that when applying rheology to study fat crystallization, the results must be interpreted with care. The application of a plate–plate geometry allowed for sensitive evaluation of the initial nucleation phase, which was not possible with an SPC. Both geometries could provide information on crystallization behavior in terms of one-step or two-step crystallization. However, in the late stage of the crystallization process, when the fat crystals form a strong network, the SPC could not describe differences in the rheology of the fat-crystal network, which was a possibility by the use of a plate–plate geometry. Thus, oscillatory rheology with a suitable geometry can be used to evaluate the entire crystallization process.

BIO PCP Ionic liquids or eutectic solvents? Identifying the metabolic distribution of vanillin in *Rhodococcus opacus* during lignin valorization

Zhou, H., *et al.*, *Bioresour. Technol.* 126348, 2021, https://doi.org/10.1016/j.biortech.2021.126348.

Vanillin bioconversion is important for the biological lignin valorization. In this study, the obscure vanillin metabolic distribution in *Rhodoccous opacus* PD630 was deciphered by combining the strategies of intermediate detection, putative gene prediction, and target gene verification. The results suggest that approximately 10% (mol/mol) of consumed vanillin is converted to vanillic acid for further metabolism, and a large amount is converted to dead-end vanillyl alcohol in *R. opacus* PD630. Subsequently, five vanillin reductases were identified in *R. opacus* PD630, among which Pd630_LPD03722 product exhibited the greatest activity. With the detected metabolic distributions of vanillin, the conversion of vanillin to muconic acid was facilitated by deleting domestic vanillin reductase genes and introducing vanillin dehydrogenase from *Sphingobium* sp. SYK-6. Ultimately, the muconic acid yield from vanillin increased to 97.83% (mol/mol) from the initial 10% (mol/mol). Moreover, this study demonstrated the existence of vanillin reductases in *Escherichia coli*, *Bacillus subtilis*, and *Corynebacterium glutamicum*.

BIO PCP Enhanced medium-chain-length polyhydroxyalkanoate production by co-fermentation of lignin and holocellulose hydrolysates

Arreola-Vargas, J., *et al.*, *Green Chem. 23*: 8226–8237, 2021, https://doi.org/10.1039/D1GC02725E.

Biological lignin conversion to medium-chain-length polyhydroxyalkanoates (mcl-PHA) has recently emerged as an attractive alternative to petroleum-based plastics due to the renewable nature of lignin and the value-added applications of mcl-PHA. Previous reports suggested that addition of limited glucose can improve mcl-PHA accumulation in *Pseudomonas putida KT2440* grown in lignin substrates. Herein, we propose a biorefinery process to systematically release lignin and sugars from lignocellulosic biomass and evaluate the potential of co-utilization of all biomass components for conversion to mcl-PHA. Our results indicate that a sequential treatment composed of acid pretreatment, enzymatic hydrolysis, and alkaline treatment produces a suitable lignin stream for mcl-PHA production in engineered *P. putida*. In addition, the PHA titer could be increased by 71% when the lignin stream (AH) was combined with the enzymatic hydrolysate (EH) at a ratio of 75 : 25. Higher ratios of EH : AH negatively affect mcl-PHA accumulation as well as mixtures with sugars from the acid hydrolysate. The optimization of the fermentation conditions, including the inoculum (OD_{600}) and substrate (soluble solid content (SSC)), was carried out by a central composite design. The optimal conditions were achieved at $OD_{600} = 2.17$ and SSC = 68.28 g L^{-1}. Under these conditions, the titer increased 4.3 fold, achieving a mcl-PHA production of 1.38 g L^{-1}. Lignin characterization before and after fermentation by nuclear magnetic resonance and gas chromatography showed that *p*-coumarates were mainly consumed during the fermentation for mcl-PHA production. Overall, this biorefinery strategy allowed us to increase mcl-PHA production by utilizing the different lignocellulosic fractions. Unlike previous studies, no model substrates were utilized at any stage of the fermentation process, representing a step forward towards process feasibility.

EAT Intermittent dynamics of bubble dissolution due to interfacial growth of fat crystals

Liascukiene, I., *et al.*, *Soft Matter 17*: 10042–10052, 2021, https://doi.org/10.1039/D1SM00902H.

Foams are inherently unstable objects that age and disappear over time. The main cause of foam aging is Ostwald ripening: Smaller air bubbles within the foam empty their gas content into larger ones. One strategy to counter Ostwald ripening consists in creating armored bubbles, where solid particles adsorbed at the air/liquid interface prevent bubbles from shrinking below a given size. Here, we study the efficiency of coating air bubbles with fat crystals to prevent bubble dissolution. A monoglyceride, monostearin, is directly crystallized at the air/oil interface. Experiments on single bubbles in a microfluidic device show that the presence of monostearin fat crystals slows down dissolution, with an efficiency that depends on the crystal size. Bubble ripening in the presence of crystals exhibits intermittent dissolution dynamics, with phases of arrest, when crystals jam at the interface, followed by phases of dissolution, when monostearin crystals are ejected from the interface. In the end, crystals do not confer enough mechanical strength to the bubbles to prevent them from fully dissolving.

H&N EAT Soybean processing wastes and their potential in the generation of high value added products

Canaan, J.M.M., *et al.*, *Food Chem. 373* : 131476, 2022, https://doi.org/10.1016/j.foodchem.2021.131476.

This study aimed to evaluate centesimal composition, microbial safety, and antioxidant activity of soybean processing wastes (okara and okara flour) and soymilk. High fiber, carbohydrate, energy, and lipids contents were found. Antioxidant activity by spectrophotometric and Electron Paramagnetic Resonance assays showed values for soybean (72.4% and 83.5%), okara (9.6% and 7.7%), okara flour (30.7% and 11.5%), and soymilk (28.4% and 36.5%). The total phenolic content was an average of 3.33 mg of gallic acid equivalent.g–1. Infrared spectra revealed no significant changes in the absorption bands, guaranteeing non-alteration in the compounds' compositions after processing. Microbiological assays indicated that soybean derivatives are safe for consumption. These results reinforce that these wastes contain bioactive compounds of interest with great potential to generate high value-added products.

LOQ EAT Combined effects of plant food processing by-products and high oxygen modified atmosphere packaging on the storage stability of beef patties

Liang, Z., *et al.*, *Food Control 133*: 108586, 2022, https://doi.org/10.1016/j.foodcont.2021.108586.

Plant food processing by-products (PFPB) are a potential source of natural antioxidants that can be used in the meat industry. This study investigated the combined effects of PFPB including sugarcane bagasse (SCF), orange peel (ORP) and tomato pomace (TMT), and high oxygen modified atmosphere packaging (HOMAP; 80 % O2: 20 % CO_2) on the oxidative and microbial stability of beef patties during cold storage for up to 12 days. The total phenolic content (TPC) and antioxidant activity of the PFPB were also evaluated. ORP was found to have the highest TPC, followed by TMT and SCF. DPPH-radical and ABTS-radical assays demonstrated a similar trend. The PFPB were proved to successfully improve the meat properties of a* value and total viable count (TVC) and minimize lipid and protein oxidation, although the effectiveness did not exceed that of butylated hydroxytoluene (BHT). The HOMAP maintained the redness of beef patties and inhibited microbial growth compared to aerobic packaging, but did not improve lipid and protein oxidative stability. Overall, the combination of HOMAP and natural antioxidants from sugarcane, tomato, and orange by-products can potentially improve the shelf life of beef patties by reducing oxidative deterioration of color, lipid, and protein, while delaying the growth of microorganisms. This research provided a reference for the future use of PFPB in meat preservation.

IOP PRO Environmental performance of microalgae hydrothermal liquefaction: life cycle assessment and improvement insights for a sustainable renewable diesel

Marangon, B.B., *et al.*, *Renew. Sustain. Energ. Rev.*, online November 19, 2021, https://doi.org/10.1016/j.rser.2021.111910.

The potential environmental impacts of bio-oil production from algal biomass via hydrothermal liquefaction (HTL) and its upgrading in renewable diesel were obtained. The gate-to-gate boundary was applied to obtain 1 MJ of energy as the functional unit. Marine eutrophication was the most impacted category due to N emission from the aqueous phase. In other categories, the HTL process was more harmful to the environment due to heat demand, and bio-oil upgrading contributed to 79% of the environmental impacts in the climate change category. The sensitivity analysis highlighted the importance of the residual heat recovery in the HTL reactor, indicating the potential to reduce up to 52% of impacts on marine ecotoxicity with a 10% increase in this parameter. Finally, a new scenario was proposed to reduce the heat input

into the HTL reactor and recirculate the aqueous phase after dilution. Results demonstrated a 45% reduction in the potential environmental impacts and a 2x greater energy balance than the base scenario. In the new scenario was emitted 0.1349 kg of CO_2 eq per MJ. In the damage assessment, the new scenario caused less damage than the base scenario, showing the benefits of the proposed improvements. When comparing the bio-oil from the new scenario with the conventional diesel, damage in the resource scarcity category was decreased by 6x. The environmental sustainability of microalgae biotechnology for bio-oil obtention through HTL still has to be improved, especially concerning subproducts valorization and heat recovery in the reaction.

PRO Comparing biofuels through the lens of sustainability: a data envelopment analysis approach

Cabrera-Jimenez, R., *et al.*, Josep M. Mateo-Sanz, Jordi Gavalda, Laureano Jimenez, Carlos Pozo, *Appl. Energy 118201*, 2021, https://doi.org/10.1016/j.apenergy.2021.118201.

Liquid biofuels can facilitate the transition toward a more sustainable transportation sector by curbing carbon emissions while maintaining most of the current vehicle fleet. Today, a myriad of alternatives are available to produce biofuels, where different decisions for the fuel type, blend, conversion process, and carbon source will affect the final cost and environmental impact of the product. In this contribution, we analyze the performance of 72 different biofuels routes based on 12 indicators that cover the three sustainability dimensions: economic, environmental, and social. The proposed multi-criteria approach combines Data Envelopment Analysis with Life Cycle Assessment to evaluate biofuels from a cradle-to-wheel perspective, that is, considering the production chain spanning from biomass production to the combustion of the biofuel in the engine. Results reveal that there are 35 biofuels routes performing better than the rest, with renewable diesel being a better option than ethanol-based blends or biodiesel, and waste biomass preferred over cellulosic biomass or bio-oils. The selection of the carbon source proofed to be the most important decision, highlighting the need to consider regional aspects related to soil and climate before promoting a certain biofuel. Overall, our results can help to derive effective policies for the adoption of biofuels attaining the best performance at minimum cost and environmental risks.

PRO ANA H&N Effects of different processing methods on the lipid composition of hazelnut oil: a lipidomics analysis

Sun, J., *et al.*, *Food Sci. Hum. Wellness 11*: 427–435, March 2022, https://doi.org/10.1016/j.fshw.2021.11.024.

Although hazelnut oil is rich in nutrients, its quality is greatly affected by how it is processed. However, no studies to date have comprehensively analyzed the lipid composition of hazelnut oil using different processing methods. Here, we conducted a lipidomics analysis using UPLC-QTOF-MS to characterize the lipid composition of cold-pressed hazelnut oil (CPO), ultrasonic-assisted hexane hazelnut oil (UHO) and enzyme-assisted aqueous hazelnut oil (EAO). A total of 10 subclasses of 98 lipids were identified, including 35 glycerolipids (GLs), 56 glycerophospholipids (GPs), and 7 sphingolipids (SPs). The total lipid and GL content were the highest in CPO, GP content was the highest in UHO, and the ceramide content in SPs was most abundant in EAO. Multivariate statistical analysis showed that the lipid profiles of hazelnut oil prepared with different processing methods varied. Twelve significantly different lipids (TAG 54:3, TAG 52:2, TAG 54:4, TAG 54:2, TAG 52:3, TAG 54:5, DAG 36:2, DAG 36:4, DAG 36:3, PC 36:2, PA 36:2 and PE 36:3) were identified, and these lipids could potentially be used as biomarkers to distinguish between hazelnut oil subjected to different processing methods. Our results provide useful information for hazelnut oil applications and new insight into the effects of edible oil processing.

PRO PCP H&N Ionic liquids or eutectic solvents? identifying the best solvents for the extraction of astaxanthin and beta-carotene from *Phaffia rhodozyma* yeast and preparation of biodegradable films

Mussagy, C., *et al.*, *Green Chem.*, online first, 2021, https://doi.org/10.1039/D1GC03521E

In an attempt to replace conventional organic solvents by more benign equivalents, such as ionic liquids or eutetic solvents, we used these alternative solvents to develop a simple and ecofriendly process for simultaneously extracting astaxanthin and beta-carotene from *Phaffia rhodozyma* biomass and preparing bioactive starch-based biodegradable films without further purification.

The use of cholinium-based eutetic solvents appeared to be a promising solution that could be used to develop functionalized carotenoids-rich biofilms.


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